2010

Anti-Diabetic Potentials of Phenolic Enriched Chilean Potato and Select Herbs of Apiaceae and Lamiaceae Families

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ANTI-DIABETIC POTENTIALS OF PHENOLIC ENRICHED CHILEAN POTATO AND SELECT HERBS OF APIACEAE AND LAMIACEAE FAMILIES

A Thesis Presented

by

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Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

SEPTEMBER 2010

MOLECULAR AND CELLULAR BIOLOGY

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DEDICATION

To

Baba, Ammi, Gulrukh, Faisal and Hassan

ACKNOWLEDGMENTS

I would like to thank Dr. Kalidas Shetty for his support and guidance throughout my career at University of Massachusetts Amherst. I would like to thank Dr. Sallie Smith-Schneider and Dr. Young-Cheul Kim for their representation of my committee.

I would like to thank my colleagues Marcia Pinto, Dipayan Sarkar and Chandrakant Ankolekar for being great mentors and guiding me throughout my Graduate career.

ABSTRACT

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SEPTEMBER 2010

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The incidence of diabetes mellitus and cardiovascular diseases is increasing at a worrisome rate globally. Diabetes mellitus is known to occur due to high blood glucose levels, caused by defects in insulin levels. Adult on-set type II diabetes, which is closely associated with obesity, is reported to be 90-95% of all diabetic cases and linked to diet and lifestyle factors. A large population of the developed and developing countries is now being effected by this epidemic. Natural sources of phenolic antioxidants and inhibitors of digestive enzymes from food sources have potential for low cost dietary management of type II diabetes. Therefore, the main focus of this study was to evaluate, develop and design effective dietary strategies based on a combination of Chilean potatoes and herb synergies for the management of hyperglycemia and hypertension linked to type II diabetes.

Antioxidant, antihypertensive and anti-hyperglycemic potentials of Chilean potato (*Solanum tuberosum ssp. tuberosum L.*), herbs from the Apiaceae (Dill, Ajowan, Fennel, Caraway, Coriander and Anise) and Lamiaceae (Sage and Marjoram) families were evaluated, with a goal to target a new dietary management strategy for early stages of

type II diabetes through lowering of hyperglycemia and related complications of hypertension

The results indicated a high correlation between total phenolic content and total antioxidant activity in several Chilean potato varieties evaluated, which indicates that certain phenolic compounds may be responsible for high antioxidant activity. Also, certain varieties of Chilean potato had antihypertensive potentials, with ACE inhibition upto 88%.

The α-glucosidase inhibition relevant for hyperglycemia management for Apiaceae family ranged upto 50% (Dill) for aqueous extracts. A high correlation ($r = 0.86$) was observed between α-glucosidase inhibition and total phenolic content for aqueous extracts of all species investigated in the Apiaceae family. A high rosmarinic acid activity was observed in aqueous extracts of Lamiaceae family, which ranged upto 39.7 mg/g of sample dry weight (DW). This suggests that high phenolic content and associated antioxidant activity found in sage and marjoram is dominated by rosmarinic acid.

High enzyme inhibitory activities, reflecting *in vitro* anti-hyperglycemic and anti-hypertensive potentials indicates that consumption of these food sources in our diet would prove to be beneficial towards our health. Further *in vivo* studies for type II diabetes-linked functionalities of these natural sources of antioxidants and inhibitors would confirm the human health benefits achieved through dietary intake.

Keywords: Angiotensin-I converting enzyme (ACE), Antioxidant Activity, α-glucosidase, Chilean potato (Solanum tuberosum ssp. tuberosum L.), Apiaceae, Lamiaceae, Phenolic Content, Rosmarinic acid

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CHAPTER 1

INTRODUCTION

Diabetes mellitus is now becoming a common metabolic disorder, resulting from the inability of our body's response to high blood glucose levels. Type II diabetes mellitus is reported to be 90-95% of all diabetic cases (Schulze and Hu, 2005). The epidemic nature of type II diabetes is closely associated with obesity, and according to World Health Organization (WHO), more than 220 million people were suffering from it in 2009 (WHO. www.who.org). The number of people projected to suffer from type II diabetes is predicted to rise over 350 million by 2030 (Diet, nutrition and the prevention of chronic diseases, www.who.org).

Remarkable progress has been achieved in development of synthetic drugs, but investigations are being carried out to discover natural and cost-effective food sources for managing hyperglycemia and hypertension associated with early stages of type II diabetes, through diets rich in legumes, fruits, vegetables, herbs and spices. These plant foods consist of basic nutrients such as vitamins, minerals, dietary fibers and more important bioactive compounds such as polyphenols and carotenoids (Montonen *et al*., 2004; Robert *et al*., 2006) that can have specific structure-function benefits (Shetty *et al*., 2008; Pinto and Shetty, 2010). Consumption of these bioactive enriched plant foods has the potential to prevent and lower the occurrence of early stages of these chronic diseases such as type II diabetes, cardiovascular diseases and their late stage complications. Many plant foods and specific varieties of specific

species contain hypoglycemic compounds, acting as antimetabolites to help block specific disease pathway, including the oxidation pathway of fatty acids (Ahmad *et al*., 2009). Plant sources utilized as medicinal herbs and seeds are known to have low toxicity levels and several therapeutic properties, due to which more recognition has been given to them as being safer than synthetic drugs. Natural plant and food sources provide α-amylase and α-glucosidase inhibitors, which plays a part in offering techniques to control postprandial hyperglycemia (Kwon *et al*., 2006; Shetty *et al*., 2008; Pinto and Shetty, 2010) with minimum side effects.

Herbs and seeds of many plant sources are being evaluated to measure their levels of phenolic phyotchemicals, containing high antioxidant activity (Kwon *et al*., 2006). Phenolic phytochemicals are used by plants to protect them from abiotic and biotic stresses, but are equally also beneficial to preventing and combating human chronic diseases linked to oxidative stress (Shetty and Wahlqvist, 2004). The presence of certain phenolic compounds in plant foods are associated and highly correlated with high antioxidant activities along with high amounts of α-amylase and α-glucosidase inhibitors, which plays a role in the treatment of managing hyperglycemia and related complications of hypertension (Kwon *et al*.,2006; Shetty *et al*., 2008; Pinto and Shetty, 2010).

Therefore the aim of this thesis was to evaluate novel Chilean potato (*Solanum tuberosum ssp. tuberosum L*.) widely used as potato-based foods in the tropical and sub-tropical regions, as well as select species of the Apiaceae and Lamiaceae families used as condiments for their anti-diabetic and anti-hypertensive potentials. A synergistic combination of the right potato varieties with the right combination of

condiments can be the basis of an effective dietary strategy for managing early stages of type II diabetes, associated hyperglycemia and its oxidation-linked micro vascular and macro vascular complications such as hypertension.

Potato originated from the Andean mountains (Andean potato – *Solanum tuberosum ssp. andigenum L.*) and also Chiloe Islands (Chilean potato – *Solanum tuberosum ssp. tuberosum L.*) of South America. Potato has been used as a food source since ancient times, but is commonly perceived as an unhealthy food item due to its high starch content and when fried with vegetable oils. It is amongst one of the most highly produced crops and the third largest food crop consumed, following rice and wheat (Camire *et al*., 2009). Phytochemicals in potato are often found in its peel, and its content is higher in cultivars with brighter peel colors (Zhang *et al.*, 2009). Phytochemicals are relevant to human health in the form of antioxidants and associate bioactive functions for specific disease conditions, and the high daily consumption of potato could contribute a high phenolic content to our diet (Xu *et al.*, 2009). Potato is known to have a low fat content, therefore its consumption in substitution for other high carbohydrate content foods such as rice and pasta may potentially benefit our overall health. Chilean potato (*Solanum tuberosum ssp. tuberosum L.*) used as genetic stock in developing tropical and sub-tropical varieties was chosen for the evaluation of its health benefits, in relation to total phenolics, antioxidant activity and *in vitro* enzyme inhibition assays (α-amylase, α-glucosidase and ACE), for potential prevention and management of early stages of type II diabetes.

Plants and especially medicinal and food herbs have been used as traditional medicine to treat common illnesses, since ancient times. Plants from the Apiaceae

family are used as food, flavoring of foods and for their medicinal purposes, such as stomachaches, abdominal pain and acidity (Shekhawat and Batra, 2006). Previous studies have suggested that species belonging to the Apiaceae family have hypoglycemic effects on humans and animals (Dhandapani *et al*., 2002). To develop treatments for prevention of chronic illnesses, this study investigated the medicinal uses and phytotherapies of seeds from select species of family Apiaceae. The discovery of innovative ideas for the development of plant-based medicine and food-based therapies would allow the availability of medicine at a lower cost compared to synthetic drugs. We evaluated the seed extracts of *Foeniculum vulgare* (Fennel), *Anethum graveolens* (Dill), *Coriandrum sativum* (Coriander), *Trachyspermum copticum* (Ajowan), *Carum carv*i (Caraway) and *Pimpinella anisum* (Anise) for their anti-diabetic potential. Evaluation of 6 select species of the family Apiaceae was done using *in vitro* assays, which provides the biochemical rationale to potentially target them for prevention of type II diabetes based on future animal and clinical studies.

Further it has been estimated that, presently, 80% of the world's population relies on the use of traditional medicine for healthcare purposes (Muthu *et al.*, 2006). Therefore, the awareness of specific herbs for medicinal use is becoming common for treating chronic illnesses. Herbs in the family Lamiaceae are known to be a rich source of phenolic antioxidants (Shetty, 1997; Kwon *et al.*, 2006), which often indicates a high correlation with α-glucosidase inhibitors and therefore have the potential for prevention of hyperglycemia. Therefore another focus of this research study was to evaluate two select species of Lamiaceae family, such as *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage), for their hypoglycemic potential.

Many plants from Lamiaceae family originated from Near East Asia, where they have been used for food preservation and treatment of common illnesses as traditional medicine and now widely used around the world (Shetty *et al.*, 1995; Kwon *et al.*, 2006). Lamiaceae family herbs contain phenolic phytochemicals, which are linked to potentially managing chronic oxidation linked diseases (Shetty, 1997), and contains potential capacity to lower mortality rates of cancer (Velioglu *et al.*, 1998). Therefore evaluating *in vitro* functionality assays would provide a biochemical rationale for the use of sage and marjoram in prevention of hyperglycemia and hypertension linked to type II diabetes.

In this thesis study, total soluble phenolics and DPPH inhibition assay to determine the total antioxidant activity and phenolic profile were evaluated to explore the potential of Chilean potato (*Solanum tuberosum ssp. tuberosum L.*), select Apiaceae family species and 2 Lamiaceae family species from the Near East region for potential use in the dietary management of early stages of hyperglycemia and hypertension. Specifically *in vitro* functionality assays such as α-amylase, α-glucosidase and angiotensin-I converting enzyme (ACE) inhibitory activities were performed to evaluate the potential of studied samples towards hyperglycemia and hypertension management.

CHAPTER 2

LITERATURE REVIEW

2.1 Hyperglycemia and Hypertension Linked to Type II Diabetes

The occurrence of type II diabetes is becoming a global epidemic which is essentially due to dramatic changes in diet and lifestyle. Diabetes is known to occur due to elevated blood glucose levels and the inability of a body's response to high blood glucose, which is caused by defects in insulin secretion, insulin action (Schulze and Hu, 2005) and several other pathological changes. Diabetes mellitus is known to be effecting people of both developed and developing countries, harming a large percentage of the world's population. Type II diabetes mellitus is reported to make up 90% - 95% of all diabetic cases. It develops when the cellular sensitivity to insulin signaling is reduced (Schulze and Hu, 2005). Type I diabetes, which is caused due to complete deficiency of insulin secretion, is due to the destruction of pancreatic beta cells. It is believed that the reason behind increase in occurrence of type II diabetes is due to environmental risk factors, and for type I diabetes there are only a few known environmental risk factors that could potentially cause the disease (Schulze and Hu, 2005). Previous studies have suggested that the adoption of Western diet (Van Dam *et al*., 2002), along with other risk factors such as obesity and the lack of physical activity are associated with increased incidence of type II diabetes.

The number of people suffering from type II diabetes is rising constantly worldwide. Due to the increase from 35 million people with type II diabetes in 1985 (Schulze and Hu, 2005) to more than 220 million people in 2009 (World Health Organization, www.who.org), we can assume that the epidemic nature of type II diabetes is due to environmental risk factors rather than genetic changes. Genetic background is unlikely to have changed the nature of type II diabetes due to the short time period. The incidence of type II diabetes is closely related to the worldwide epidemic of obesity, and the amount of people projected to have type II diabetes is predicted to increase over 350 million by 2030 (Diet, nutrition and the prevention of chronic diseases, www.who.org).

Cardiovascular diseases (CVD) are associated with type II diabetes as one of its main consequence, and majority of people effected with type II diabetes die of cardiovascular complications (Schulze and Hu, 2005). The risk of cardiovascular diseases in patients with type II diabetes is 2-6 times higher than people without type II diabetes (Gaede *et al*., 2003). Type II diabetes associated cardiovascular diseases and high incidence of micro vascular and macro vascular complications is also the leading cause of blindness, kidney failure and amputations (Gaede *et al*., 2003; Schulze and Hu, 2005). The direct medical cost of diabetes and diseases linked with diabetes is enormous, e.g., it was estimated to be over $132 billion in 2002 (Report from the American Diabetes Association, 2002). The rise in economic spending for type II diabetes is going to rise enormously, since the World Health Organization predicted that there is going to be over 350 million people affected with type II diabetes by 2030.

There is no known cure for diabetes. Therefore bringing out awareness for changing the diet and lifestyle through which the occurrence of such illnesses could be reduced is the best strategy. There has been a remarkable progress and development in the therapy of type II diabetes mellitus through synthetic drugs. However, the investigation for anti-diabetic substances from natural sources for management of hyperglycemia and hypertension linked to type II diabetes is being pursued due to their lower toxicity and side effects (Ahmad *et al.*, 2009; Pinto and Shetty, 2010).

Plant-based foods used for medicinal therapies have been used since ancient times to treat common illnesses. Recent studies have found activities of various plant foods containing anti-metabolites that help prevent the oxidation pathway of fatty acids (Ahmad *et al*., 2009), which has consequences for managing hypoglycemia. Natural α-amylase and α-glucosidase inhibitors from food-grade plants provide dietary strategies to control postprandial hyperglycemia, and the natural form of these inhibitors could be used in therapies with minimum side effects (Kwon *et al.*, 2006; Shetty *et al*., 2008; Pinto and Shetty, 2010). Therefore, food-based biochemical studies on bringing awareness towards managing hyperglycemia and hypertension linked to type II diabetes through dietary management is gaining enormous importance. The inclusion of diets rich in fruits and vegetables compared to diets including red meat and sweets, independent of physical activity and family history of diabetes, indicates a lower risk for type II diabetes (Van Dam *et al*., 2002). Therefore, the diets rich in fruits and vegetables would also indicate a lower risk for

cardiovascular complications, due to the high phenolic antioxidants and natural enzyme inhibitors.

2.2 Chilean Potato (*Solanum tuberosum ssp. tuberosum L.*)

Potato belongs to the *Solanaceae* family, and originated from the Andean Mountains (Andean potato – *Solanum tuberosum ssp. andigenum L.*) and Chiloe Islands (Chilean potato – *Solanum tuberosum ssp. tuberosum L.*) of South America, where it has been used as a food source for thousands of years (Raker and Spooner, 2002; Ames and Spooner, 2008). Potatoes (*Solanum tuberosum ssp. tuberosum L.*) grown in the Chilean region, as well as from the region of Venezuela and Argentina (*Solanum tuberosum ssp. andigenum L.*) are known as high yielding crops (Ames and Spooner, 2008). It has been known that Chilean potato is evolved from crossing of subspecies *andigenum* and an unidentified wild species (Grun, 1990; Raker and Spooner, 2002). There have been no studies carried out to explore the potential benefits of Chilean potato, in preventing and managing hyperglycemia and hypertension linked to type II diabetes.

Potato (*Solanum tuberosum L.*) is a highly produced food crop in the world, and the third largest food crop consumed, following rice and wheat (Camire *et al.*, 2009). Potato is commonly believed to be unhealthy due to its high starch and high consumption when fried with oil, and many people are not aware of its health benefits. Often, it is believed that potato consists of high caloric and fat content compared to rice; which is incorrect since potato has a low fat and energy density,

similar to legumes (Priestly, 2006; Camire *et al.*, 2009). Consumption of potato in the right amount, right high bioactive variety and with low processing could potentially lead to the prevention of oxidation-linked chronic diseases such a type II diabetes and cardiovascular diseases as opposed to its usual form of French fries and potato chips.

Fruits and vegetables consist of nutrients such as vitamins, minerals and dietary fibers, as well as other bioactive compounds such as polyphenols and carotenoids (Montonen *et al.*, 2004; Robert *et al.*, 2006), which are beneficial towards human health (Shetty *et al*., 2008). Consumers are becoming more aware of the nutritional benefits such as high antioxidant activities from fruits and vegetables than ever before. Phytochemicals and specifically secondary metabolites like phenolics are produced by plants to protect themselves from abiotic and biotic stresses, but they could also potentially prove to be beneficial to manage human chronic diseases induced under oxidative stress for high calories and life style changes (Shetty and Wahlqvist, 2004).

The peel of potato is a good source of phytochemicals such as anthocyanins, and its content is higher in potatoes with brighter peel colors (Zhang *et al.*, 2009). Anthocyanins are also known to have other biological functions such as high antioxidants, antimicrobial and anti-obesity capacities (Zhang *et al.*, 2009), and the presence of anthocyanins in potato peel is 3-4 times higher than in the tuber. Phytochemicals are associated to human health in the form of antioxidants, and are associated with disease prevention abilities. Potato is usually not considered as a food item with high antioxidant activity, but due to its high daily consumption, it is known to contribute high total phenolic content to our diet (Xu *et al.*, 2009). The high

amount of antioxidants consumed through inclusion of potato in out diet could be related to efficiently helping defend our bodies from cardiovascular diseases, by limiting oxidative stress (Chu *et al.*, 2002; Robert *et al.*, 2006).

A important vitamin obtained from fruits and vegetables beneficial for human health is vitamin C, and potato is rich in vitamin C with the content of 15 mg/100 g of steamed potato (Robert *et al.*, 2006). The amount of vitamin C in a potato contributes to 25-30% of our daily recommended dietary allowance (Robert *et al.*, 2006). Pinto *et al* (2008), suggested that the presence of α-glucosidase inhibitors from certain plant foods contributes to management of hyperglycemia linked to type II diabetes. Since 90% of the diabetic patients are suffering from type II diabetes, it is important that dietary α-glucosidase inhibitors are present, to prevent the absorption of glucose in small intestine (Ranilla *et al.*, 2010). Therefore, type II diabetes could potentially be controlled via inhibition of α-glucosidase enzyme, which participates in digestion of carbohydrates. Potato varieties that have high α-glucosidase inhibitors can further be used *in vivo* experiments as part of a therapeutic strategy, for management of hyperglycemia linked to type II diabetes. Another inhibitor related to α-glucosidase inhibitor is α-amylase inhibitor, which is also known to play an important role in the management of hyperglycemia linked to type II diabetes (Pinto *et al.*, 2009). The inhibitory activities of these two enzymes are often linked to certain phenolic compounds present in plant foods. Therefore, the inhibition of these enzymes could vary by being high or low depending on the presence of phenolic phytochemicals that enhance their activities in species specific plant species and varieties (Kwon *et al*., 2006; Pinto *et al.*, 2009). It has been suggested that inhibition of both α-glucosidase

and α-amylase necessarily does not have to be present to help manage hyperglycemia, as long as one of the inhibitor types show a high activity (Pinto *et al.*, 2009). However foods with high α-glucosidase inhibitor and low or medium α-amylase inhibitor are considered ideal to avoid digestive complications from undigested starch (Kwon *et al*., 2006).

Cardiovascular diseases are one of the leading causes of death in the world, and type II diabetes increases the susceptibility to cardiovascular diseases due to micro vascular and macro vascular complications. Diets rich in fruits and vegetables are recommended for hypertension management, along with hyperglycemia linked to type II diabetes (Bazzano *et al*., 2003). The combination of micronutrients, antioxidants, phytochemicals and fibers present in fruits and vegetables also help reduce the overall risk of cardiovascular diseases (Liu *et al.*, 2000). An important target for managing vascular complications includes angiotensin converting enzyme inhibition, which is used for treating hypertension associated with congestive heart failures. Angiotensin I-converting enzyme (ACE) regulates vascular hypertension by two different reactions (Johnston, 1992; Pinto *et al.*, 2009). One way is to convert angiotensin I into a vasoconstrictor, angiotensin II, and vasodilator bradykinin that helps lower blood pressure (Johnston, 1992).

The different phenolic compounds from potato could be correlated with high or low ACE inhibitory activity. This would indicate the potential of regular intake of phenolic compounds through our diet for beneficial effects on cardiovascular system. Chlorogenic acid forms 90% of the total phenolic compounds in potato tuber (Dao and Friedman, 1992), which ensures the quality and safety of potato plant.

Chlorogenic acid has the ability to lower blood pressure in hypertensive patients, and its derivative has shown to lower blood pressure in hypertensive rats (Cheplick, 2010). Potato is also a good source of ferulic acid, supplying 5-70 mg/ 100 g, and a source of dietary antioxidant to prevent vascular complications and type II diabetes (Zhao *et al*., 2008). Another compound often observed in potatoes include caffeic acid, which is a natural antioxidant abundant in plant foods, and has been proven to be beneficial in cardiovascular complications by indicating hypotensive effects in hypertensive rats (Li *et al.*, 2005). Catechin activity in potato indicates the prevention of type II diabetes (Matsui *et al.*, 2007), cardiovascular complications and cancer prevention abilities (Nagao *et al*., 2007). The inclusion of catechins through consumption of potato, especially in high amounts is known to reduce fat, cholesterol levels and blood pressure (Nagao *et al*., 2007).

Therefore, the inclusion of whole potato in our diet, not in its usual form of French fries and potato chips, has potential health benefits by managing hyperglycemia and hypertension linked to type II diabetes.

2.3 Apiaceae (Carrot) Family

The use of plant food sources and especially food herbs in traditional medicine has increased the interest in exploring their beneficial effects on human health. An estimated 70% of population globally utilizes traditional medicine obtained from plants to treat and cure various ailments (Jiofack *et al*., 2009). Plants from the family Apiaceae (Carrot) are commonly used as food, flavoring of foods and

for their medicinal purposes. In particular, the seeds from family Apiaceae are known to be used as a household remedy for complications such as hypertension (Gilani *et al*., 2005). In other regions of the world such as India, plants from family Apiaceae are used to treat common ailments such as stomachaches, abdominal pain and acidity (Shekhawat and Batra, 2006). In India, a mixture of various plants is often consumed orally with water, or applied externally by massaging the abdomen with it (Shekhawat and Batra, 2006). In other parts of the world such as Cameroon, the use of plants from Apiaceae family are targeted in therapies for vomiting, appendicitis, ingestion, constipation and to treat mosquito bites (Jiofact *et al.*, 2009).

Another ancient form of traditional medicine is known as 'Ayurveda', which uses the active ingredients from plants in treating various illnesses (Dhandapani *et al.*, 2002). The use of plants is due to their less toxic, as well as more free from side effects compared to synthetic products. Several plants belonging to the Apiaceae family are considered to have hypoglycemic effects on humans and animals (Dhandapani *et al.*, 2002). The hypoglycemic effects of plants could possibly be due to insulin affect, either by increasing pancreatic secretion of insulin from the cells of islets of Langerhan's or its release from bound insulin (Prasanna, 2000; Dhandapani *et al*., 2002).

Various beneficial effects have been observed for seed extracts belonging to the Apiaceae family, and following are the select species evaluated. Essential seed oils form *Foeniculum vulgare* (Fennel) such as anethole and limonene are used for medicinal purposes, and the seeds are also used as tranquilizers and tonics (Oktay *et al.*, 2003). Aqueous extracts of fennel seeds are observed for their hypotensive effects

in a dose related manner (Oktay *et al.*, 2003). *Anethum graveolens* (Dill) and *Trachyspermum copticum* (Ajowan) seed extracts have been used to treat diarrhea, ingestion and common colds (Husain *et al*., 2008), and Dill is also fed to cows and goats for improving milk production (Lans *et al.*, 2007). Ajowan seed oils have been suggested to treat medical problems affecting joints or painful muscle conditions (Husain *et al.*, 2008). *Coriandrum sativum* (Coriander) seeds are often used as food flavoring agent and to treat ulcers (Husain *et al.*, 2008). *Carum carvi* (Caraway) plant is beneficial in treatment and management of type II diabetes and cardiovascular diseases, and evokes beneficial effects on elevation of lipids in the bloodstream (Lemhadri *et al*., 2006). *Pimpinella anisum* (Anise) belongs to the Middle Eastern region, where it is used as an aromatic spice and to help in digestion (Arslan *et al*., 2004).

Phenolic antioxidants are attributed to the therapeutic use of medicinal plants in managing hyperglycemia, by playing a part in causing delay in development of type II diabetes. High content of polyphenols and vitamins due to high total phenolic and total antioxidant activity, gives an excellent rationale for using plant sources for medicinal purposes. It has been suggested that the high antioxidant activity potential is often due to certain phenolic compounds (Kesilova *et al*., 2006). The usefulness of antioxidants in our diet could be described by slowing down the oxidation of fats (Yen and Duh, 1994), as well as being identified as free radicals or active oxygen scavengers (Oktay *et al.*, 2003). The inclusion of antioxidants in our diet by various food sources could lead to weight loss, controlling obesity which is linked to type II diabetes. Natural sources of antioxidants have the capability to protect against free

radicals and chronic diseases (Oktay *et al.*, 2003), whereas synthetic sources of antioxidants are restricted due to their carcinogenicity (Zheng and Wang, 2001).

The use of natural herbal medicine for the prevention of blood pressure and type II diabetes is practiced worldwide (Loizzo *et al*., 2008). The α-glucosidase inhibition for select species of family Apiaceae suggests that, it would delay the degradation of oligosaccharides, decreasing the absorption of glucose, which would inhibit the increase in postprandial hyperglycemia (Loizzo *et al.*, 2008). The high α-glucosidase inhibitory activity is often associated with high total phenolic content and antioxidant activity, which suggests that certain phenolic compounds are responsible for this action. Compared to α-glucosidase inhibitory activity being associated with either total phenolic content or antioxidant activity, it has been suggested that high α-amylase inhibition is not linked to either of these activities (Cheplick *et al.*, 2010). Excess α-amylase inhibition could lead to stomach distention and discomfort, even though α-amylase inhibitory activity has positive effects on prevention of hyperglycemia, linked to type II diabetes (Cheplick *et al.*, 2010). Since Apiaceae family consists of moderate α-amylase inhibition and good α-glucosidase inhibition, it is considered a good candidate for managing early stage hyperglycemia linked to type II diabetes.

Hypertension is a known risk factor for various vascular complications, associated with long term diabetes (Kwon *et al.*, 2006). Type II diabetes plays a part in elevating plasma lipids, which serves as a risk factor for coronary heart diseases (Chatterjea and Shinde, 1994; Dhandapani *et al.*, 2002). The risk of vascular complications could be

decreased by lowering of plasma lipid levels by dietary strategies (Kanne and McGee, 1976; Scott, 1999; Dhandapani *et al.*, 2002).

ACE inhibitory activity was not indicated for the family Apiaceae, which could be due to the absence of certain phenolic compounds. Phenolic profile analysis by HPLC helps recognize the phenolic phytochemicals in certain foods. Caffeic acid is known to be present in fruits and vegetables, and have anti-inflammatory and antioxidant properties (Son and Lewis, 2002). The presence of this compound in select species of Apiaceae family suggests its disease prevention potential for type II diabetes and vascular problems. The anti-cancer and antioxidant activities of phenolic compound, catechin, has attracted attention due to its benefits on human health. It is known that the ingestion of catechins is supposed to decrease waist size while reducing body fat (Nagao *et al.*, 2007), and helping to slow down the incidence of type II diabetes by managing hyperglycemia. Rutin content is high in Apiaceae family, and it is known to have special effects on dilating blood vessels and improving interpenetration of veins (Wand *et al.*, 2003). Rutin is a compound found in many kinds of plants, and it is reported to consist of anti-inflammatory and antioxidant activities. Its antioxidant activity is accountable for preventing oxidative stress in pancreatic beta cells (Heineke *et al.*, 1993), (preventing the uncontrolled proliferation of damaged pancreatic beta cells, not resulting in diabetes). The presence of rosmarinic acid suggests that it could be targeted against the production of oxidation-linked diseases (Shetty and Wahlqvist, 2004). The presence of all the above phenolic compounds indicates that Apiaceae family has the ability to provide protection against oxidation linked diseases.

Food designs and medicinal formulations using seeds from Apiaceae family and incorporating them into the use for therapeutic purposes would prove to be beneficial, due to its hyperglycemic inhibitory activities, linked to type II diabetes.

2.4 Lamiaceae (Mint) Family

Many plants related to Lamiaceae family have been originated from the Middle Eastern region in Asia. *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage) belongs to Lamiaceae (Mint) family, and also includes plants such as rosemary and lavenders. *Salvia* is genera of plants considered to be widely diverse in Turkey, and used as traditional medicine to treat common colds and stomach disorders (Cuvelier *et al.*, 1996). Water extracts of *Salvia libanotica* (Sage) in Middle East are used to treat common colds, coughs and as anti-inflammatory agents in the oral cavity (Farhat *et al.*, 2001). Also, *Origanum majorana* (Marjoram) is rich in essential oils, characterized for high amount of phenolic compounds. In Middle East, marjoram is used as folk medicine particularly in the form of tea, prescribed for fever, sinus congestion, as well as used to treat nervous disorders (Qari, 2008). Previous studies have indicated that essential oils from sage and marjoram have antibacterial, antimicrobial and suppressive activities against tumor formation (Hilan *et al.*, 1997; Farhat *et al.*, 2001). Marjoram is important as it consists of anti-hepatoma and anti-genotoxicity activities; reducing the number of cell and chromosomal aberrations (Qari, 2008). The characteristics of sage and marjoram observed through various

studies have determined their importance in preventive role for managing type II diabetes.

Herbs from Lamiaceae family are also commonly used for food preservation, culinary flavors and treatment of common illnesses as traditional medicine (Kwon *et al.*, 2006). Lamiaceae herbs are rich sources of phenolic phytochemicals and antioxidants (Kwon *et al*., 2006); which are highly correlated with α-glucosidase inhibitors, playing a potential role in hyperglycemia management. Plants belonging to Lamiaceae family are great sources of natural antioxidants, often used as spices and aromatic herbs. Therefore phenolic phytochemicals from Lamiaceae family in general have potential for managing chronic oxidation linked complications, such as cardiovascular diseases and type II diabetes (Shetty, 1997; Kwon *et al*., 2006). The antioxidant activity associated with phytochemicals is also linked potentially to lowering mortality rates of cancer in humans (Veioglu *et al.*, 1998).

When considering suitable extracts, possible reason for higher phenolic content determined for aqueous extracts is due to high temperature during sample preparation, compared to ethanolic extracts (Seaberg *et al*., 2003; Chun *et al*., 2005). High concentration of phenolic phytochemicals observed for oregano confirms its high antioxidant and antimicrobial activity (Chun *et al.*, 2005). High antioxidant activity associated with total phenolic content, allows the consumption of sage and marjoram in our diet to delay, or prevent the oxidation of lipids by inhibiting the propagation and oxidizing chain reaction (Zheng and Wang, 2001; Chun *et al*., 2005). The dietary phenolic antioxidants found as natural food sources, are important for delaying the development of chronic diseases, such as cardiovascular complications and cancers

(Shetty, 1997; Akyon, 2002; Chun *et al.*, 2005). The antioxidant activity of phenolic compounds may not be mainly due to phenolic content, but may be due to the redox properties, physico-chemical structure and nature of the individual phenolics (Kahkonen *et al*, 1999; Zheng and Wang, 2001; Parejo *et al.*, 2002; Chun *et al., 2005*). The potential of plant-based additives from sage and marjoram to prevent oxidative stress proves that, the substitution of natural plant extracts for synthetic source of antioxidants also have the potential to influence human health (Hinneburg *et al.*, 2006; Martinez-Tome *et al.*, 2001). These herbs have the potential to be industrially efficient due to their high phenolic antioxidants, slowing down the oxidative degradation of lipids (Wojdylo *et al.*, 2007), and improving the quality and nutritional value of food. Pancreatic beta cells are damaged due to oxidative stress before they are proliferated. If damaged pancreatic beta cells are prevented from proliferating through cell repair or apoptosis, the possibility of incidence of diabetes could be reduced. The high antioxidant activity potentially suppresses the oxidative stress caused to pancreatic beta cells, reducing the risk of diabetes (Song *et al.*, 2005; Bhandari *et al.*, 2008). Therefore, plants from Lamiaceae family with high phenolic antioxidants could prevent the occurrence of diabetes, applied through dietary management strategies in early stages.

The incidence of type II diabetes is growing at a worrisome rate, mainly due to diet and lifestyle adopted in the developed and developing countries. Therefore, therapeutic approaches including food based plants as medicinal sources are being developed for prevention of chronic illnesses. Decreasing the risk of postprandial hyperglycemia through a therapeutic approach can be achieved by slowing the

absorption of glucose, by inhibiting α-glucosidase enzyme in the digestive organs (Bhandari *et al*., 2008). Lamiaceae herb samples containing certain phenolic compounds such as rosmarinic acid, caffeic acid and rutin may have hypoglycemic effects, due to high α-glucosidase inhibitory activities. The consumption of Lamiaceae herbs as condiments would result in enhancement of our health, due to their potential health benefits in terms of α-glucosidase inhibition relevant to hyperglycemia linked to type II diabetes. Inclusion of these herbs in our diet would potentially reduce blood glucose concentration and lengthen the duration of carbohydrate absorption (Ye *et al*., 2002). This would serve to be important because lowering of the blood glucose level to normal is the most important part of treating persistent hyperglycemia, which is the characteristic of diabetes (Ye *et al*., 2002).

Hypertension is a risk factor for formation of cardiovascular complications, related to long term diabetes (Kwon *et al.*, 2006) and was investigated to explore the Lamiaceae species in this study. Further the phenolic compound often found to be abundant in plant sources, especially in Lamiaceae family is rosmarinic acid (Peterson and Simmonds, 2003) and could indirectly benefit hypertension management. Rosmarinic acid contains high antioxidant activity, providing protection against oxidation linked illnesses in general (Peterson and Simmonds, 2003), which could be the reason for high antioxidant activity generally seen for sage and marjoram. The presence of rosmarinic acid in plants belonging to Lamiaceae family would prevent and manage hyperglycemia associated and linked to obesity. Further *in vivo* studies would be essential for understanding the benefits of consuming rosmarinic acid-enriched herbs on human health. Caffeic acid is a phenolic compound

which plays an important part in fruits and vegetables as an antioxidant. Rutin is a phenolic flavonoid found in many fruits and vegetables, which is used to provide protection against development of vascular diseases (Schramm and German, 1998). The presence of rutin would give us some evidence that targeting sage could be used to benefit human health by managing hypertension, linked to type II diabetes.

Clinical information on the functions of sage and marjoram from Lamiaceae family could be further applied *in vivo* studies for development of innovative ingredient designs and formulations, for therapeutic strategies, to prevent chronic illnesses associated with type II diabetes and its complications.

CHAPTER 3

OBJECTIVES

3.1 Chilean Potato (*Solanum tuberosum ssp. tuberosum L.*)

(a) To study the phenolic-linked anti-diabetic potential of 54 sub-tropical cultivars of Chilean potato.

(b) Study the α-Glucosidase, α-Amylase and Angiotensin-I Converting Enzyme (ACE) inhibitory activities.

(c) To screen antioxidant, anti-hypertensive and anti-diabetic potentials.

(d) Determine functionalities of potato for better dietary management and future cultivar recommendation for potentially managing early stage type II diabetes through diet.

3.2 Apiaceae (Carrot) Family

(a) To study the medicinal uses and phytotherapies of six select species from Apiaceae family with a focus on their seeds.

(b) To investigate the functionalities of select species and how their *in vitro* effects could provide the biochemical rationale to potentially target them towards prevention of type II diabetes.

(c) Evaluate total soluble phenolics and DPPH inhibition assay to determine the total antioxidant activity and phenolic content of seeds.

(d) Study *in vitro* assays such as α-glucosidase, α-amylase and ACE inhibitory activities to evaluate seeds potential treatment of hyperglycemia and hypertension management.

3.3 Lamiaceae (Mint) Family

(a) To screen two species (*Origanum majorana* and *Salvia libanotica*) belonging to the Lamiaceae family from Near East Asia.

(b) Evaluate *in vitro* assay such as total soluble phenolic and inhibitory assays such as DPPH, α-glucosidase, α-amylase and ACE.

(c) To provide the biochemical rationale for use of sage and marjoram in prevention of hyperglycemia and hypertension linked to type II diabetes.

(d) To determine individual phenolic compounds found through HPLC analysis of phenolic phytochemicals, allowing us to correlate the phenolic compounds of the herbs to total antioxidant activity and total phenolics content.

CHAPTER 4

ANTI-HYPERTENSIVE AND ANTI-HYPERGLYCEMIC MANAGEMENT

4.1 Evaluation of Cultivars of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.)* for Diabetes and Hypertension Management Potential Using *In Vitro* Models

4.1.1 Abstract

The rate of type II diabetes is increasing globally at a worrisome rate, especially due to high rates of obesity. Water extracts of 54 different Chilean potato (*Solanum tuberosum ssp. tuberosum L.)* varieties were evaluated for total phenolics and antioxidant activity by 2, 2-diphenyl-1-picrydrazyl (DPPH) radical scavenging assay. *In vitro* functionality assays such as inhibition of α-glucosidase and α-amylase relevant for anti-hyperglycemia potential and angiotensin I-converting enzyme (ACE) for anti-hypertension potential were evaluated. The total phenolic content ranged from 0.92 mg/g (PA 43) to 13.1 mg/g (PA 4) of sample dry weight. A high correlation ($r = 0.83$) was observed for total phenolics and antioxidant activity by DPPH assay. Moderate activity of α-glucosidase inhibition was observed in several varieties with no α-amylase inhibitory activity. The anti-hypertensive potential of Chilean potato was high in select varieties, with the line PA 17 having 88% ACE inhibition. These *in vitro* assays indicate that sub-tropical cultivars of Chilean potatoes have anti-hyperglycemia and anti-hypertensive potentials, which could be part of breeding lines to develop whole potato food products for effective diet designs. These diet designs based on animal and clinical studies can be

targeted for managing early stages of type II diabetes when glucose management and exercise can help better manage overall state of this chronic disease.

4.1.2 Practical Applications

In this study, we evaluated 54 sub-tropical Chilean potato (*Solanum tuberosum ssp. tuberosum L.)* varieties to determine their relevance in managing hyperglycemia and hypertension relevant for type II diabetes using *in vitro* models. This study established that some Chilean potato varieties have the potential to manage hypertension and hyperglycemia. The whole potato food products from such varieties could be part of food designs and could be part of effective breeding programs to develop potato varieties with health potential. This research gives us biochemical rationale for further *in vivo* animal and clinical studies based on the *in vitro* enzyme assay models. This study can potentially provide us with strategies to develop new food designs based Chilean potato breeding stocks which can be parts of diverse diet designs with more effective potential for reducing hypertension and hyperglycemia linked to type II diabetes.

4.1.3 Introduction

Consumption of diets rich in fruits and vegetables are associated with lower occurrence of type II diabetes and cardiovascular diseases, which are supported by several biological and nutritional reasons. Fruits and vegetables consist of nutrients such as vitamins, minerals and dietary fibers, as well as other bioactive compounds such as polyphenols and carotenoids with potential health benefits (Montonen *et al*., 2004; Shetty and Wahlqvist, 2004; Robert *et al*., 2006; Shetty et al., 2010).

Several studies have indicated that the current global diabetes epidemic is due to dramatic changes in diet and lifestyle. Diabetes results from elevated blood glucose levels which are caused by defects in insulin production and secretion in response to glucose from dietary sources. Type II diabetes is reported to be 90%-95% of all diabetic cases, and it develops when the production of insulin is insufficient to overcome the underlying abnormality of increased glucose from the diet leading to increased resistance to its action (Schulze and Hu, 2005)). According to World Health Organization, in 2009 more than 220 million people were affected with type II diabetes, and the diabetes epidemic is closely related to the worldwide epidemic of obesity (World Health Organization, www.who.org). The 220 million people currently living with type II diabetes is predicted to increase to over 350 million by 2030 (Diet, nutrition and the prevention of chronic diseases, www.who.org).

Potato belongs to the *Solanaceae* family and originated from the Andean mountains (Andean potato - *Solanum tuberosum ssp. andigenum L.)* and Chiloe Islands (Chilean potato - *Solanum tuberosum ssp. tuberosum L)* of South America, where it has

been used as a food source for thousands of years. It is commonly believed that potato is unhealthy due to its high starch content and oil content when fried, and many people are not aware of its health benefits from a range of other nutritional compounds such as vitamins and bioactive phenolics., Potato is known to be one of the highly produced starch crops in the world for quick source of calories, and third largest food crop consumed in the world following rice and wheat (Camire *et al*., 2009). Potatoes are a good source of starch when consumed in whole food form with energy density similar to legumes (Camire *et al*., 2009; Priestly, 2006) with additional potential from bioactive phenolic antioxidants.

Today, consumers are more aware of health benefits of antioxidants from fruits and vegetables than ever in addition to their nutritional benefits from minerals and vitamins. Phytochemicals and secondary metabolites like phenolics are produced by plants to protect it from abiotic and biotic stresses, but they are also beneficial to humans under disease induced oxidative stress (Shetty and Wahlqvist, 2004). Anthocyanins are phenolic phytochemicals that are typically found in the peel of potatoes, and its content is higher in cultivars that consist of brighter peel colors (Zhang *et al*., 2009). Antoxidant phytochemicals are known to have disease prevention abilities which are associated with overall human health. Generally, potato is not considered a high antioxidant containing food based on refined starch products, but due to its high daily consumption from whole food form and products it is known to contribute high total phenolic content to our diet (Xu *et al*., 2009).

Increased carbohydrates and its conversion to fat in addition to fat intake have been suggested to promote weight gain and obesity, contributing to type II diabetes. High

fat intake in diet may also cause insulin resistance, independent of obesity (Diet, nutrition and the prevention of chronic diseases, www.who.org). Addition of potatoes in whole food with low deep frying to our diet compared to other high content carbohydrate foods such as rice and pasta may benefit our health due to its lower fat content. In addition factors such as enhanced physical activity with reduced smoking and consumption of alcohol are known to reduce the risk of type II diabetes by maintaining a healthy body weight and lifestyle.

Therapeutic and clinical strategies that manage type II diabetes is through the reduction of glucose by decreasing starch hydrolysis via blocking the pancreatic α-amylase (Pinto *et al*., 2009). Also, α-glucosidase enzyme is used to prevent intestinal glucose absorption with dietary strategies being potentially safer (Kwon *et al*., 2006). The natural forms of α-glucosidase and α-amylase inhibitors that we ingest through our daily diet have potential to prevent hyperglycemia helping manage the incidence of type II diabetes. Angiotensin I-converting enzyme (ACE) regulates vascular hypertension via two different reactions (Johnston, 1992; Pinto *et al*., 2009). One way is to convert angiotensin I into a vasoconstrictor, angiotensin II, and vasodilator bradykinin that helps in lowering blood pressure (Johnston, 1992). Hypertension could be enhanced due to occurrence of type II diabetes; therefore, the inhibition of ACE could be helpful in decreasing hypertension linked to complications of type II diabetes. Therefore, the main objective of this study was to evaluate health benefits of Chilean potato (*Solanum tuberosum ssp. tuberosum L.),* in relation to total phenolics, antioxidant activity by DPPH and *in vitro* enzyme inhibition (α-amylase, α-glucosidase, and ACE) for potential

reduction of hyperglycemia and hypertension for overall dietary management of type II diabetes.

The phenolic-linked anti-diabetic potential of 54 sub-tropical cultivars (varieties) of Chilean potato was evaluated. The screening of health-linked functional potential in these potato cultivars can help us to make cultivar recommendation and better varieties for potentially managing early stage type II diabetes through diet.

4.1.4 Materials and Methods

4.1.4.1 Materials

Dried samples of 54 different cultivars of sub-tropical Chilean potatoes (*Solanum tuberosum ssp. tuberosum L.)* were received from Puerto Montt, Chile. Porcine pancreatic α-amylase (EC 3.2.1.1), rat intestinal α-glucosidase (EC 3.2.1.20), hippuric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), rabbit lung ACE (EC 3.4.15.1), cinnamic acid, rosmarinic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO).

4.1.4.2 Sample Preparation

Water Extracts:

The samples (2.5 g) were extracted in 100 mL of distilled water under reflux at 95°C for 30 minutes. The samples were centrifuged for 10 minutes.

4.1.4.3 Total Soluble Phenolic Assay

The total phenolics in all samples were determined by using a method modified by Shetty *et al.* (1995). In brief, 0.5 mL of sample extract was added to a test tube and mixed with 0.5 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (vol/vol) Folin-Ciocalteu reagent was added and mixed. The absorbance was read

at 725 nm using a spectrophotometer (Genesys UV/Visible, Milton Roy, Inc., Rochester, NY). Different concentrations of gallic acid were used to develop a standard curve. Results were expressed as mg of gallic acid/g of sample dry weight (DW).

4.1.4.4 Total Antioxidant Activity by DPPH Radical Inhibition Assay

The antioxidant activity was determined by the DPPH radical scavenging method modified from Kwon *et al.* (2006). A 250 μL aliquot of the sample extract was mixed with 1,250 μL of DPPH (60 μM in ethanol). The mixture was centrifuged at 13,000 *g* for 1 minute, and after this the absorbance was measured at 517 nm using the Genesys UV/Visible spectrophotometer. The readings were compared with the controls, containing 95% ethanol instead of sample extract. The percentage inhibition was calculated by:

$$\%\,\text{inhibition} = \frac{(Absorbance_{\text{control}} - Absorbance_{\text{extract}})}{Absorbance_{\text{control}}} \times 100$$

4.1.4.5 α-Amylase Inhibition Assay

The α-amylase inhibitory activity was determined by an assay modified from the *Worthington Enzyme Manual* (Worthington, 1993). A total of 500 μL of sample extract and 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25°C for 10 minutes. After

preincubation, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction was stopped with 1.0 mL of dinitrosalicylic (DNS) acid color reagent. The test tubes were incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted after adding 5-15 mL of distilled water, and the absorbance was measured at 540 nm using the Genesys UV/Visible spectrophotometer. The readings were compared with the controls, containing buffer instead of sample extract. The percentage α-amylase inhibitory activity was calculated with the same equation as for percentage inhibition in the DPPH radical inhibition assay.

4.1.4.6 α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was determined by an assay modified from McCue *et al*. (2005). α-Glucosidase was assayed by using 50 μL of sample extracts and 100 μL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 U/mL) and was incubated in 96-well plates at 25°C for 10 min. After preincubation, 50 μL of 5 mM p-nitrophenyl-α-d-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, absorbance readings were recorded at 405 nm by a microplate reader (Thermomax, Molecular Devices Co., Sunnyvale, CA) and compared to a control that had 50 μL of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated with the same equation as for percentage inhibition in the DPPH radical inhibition assay. Dose

dependency was tested using 25 µL and 10 µL of the sample, the volume made up to 50 µL using 0.1 M phosphate buffer (pH 6.9) and same protocol was followed.

4.1.4.7 ACE Inhibition Assay

ACE inhibition was assayed by a method modified by Kwon *et al.* (2006). The substrate hippuryl-histidyl-leucine (HHL) and the enzyme ACE-I from rabbit lung (EC 3.4.15.1) were used. Fifty µL of sample extracts were incubated with 100 µL of 1 M NaCl-borate buffer (pH 8.3) containing 2 mU of ACE-I solution at 37°C for 10 min. After preincubation, 100 µL of a 5 mU substrate (HHL) solution was added to the reaction mixture. Test solutions were incubated at 37°C for 1 hour. The reaction was stopped with 150 µL of 0.5 N HCl. Five µL of the sample was injected in a high-performance liquid chromatography (HPLC) apparatus (Agilent 1100 series equipped with autosampler and DAD 1100 diode array detector, Agilent Technologies, Palo Alto, CA). The solvents used for gradient were (1) 10 mM phosphoric acid (pH 2.5) and (2) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for 5 min and then was decreased to 0% for the next 5 min (total run time, 18 min). The analytical column used was an Agilent Nucleosil 100-5C18, 250 mm × 4.6 mm inside diameter, with packing material of 5 µm particle size at a flow rate of 1 ml/min at ambient temperature. During each run, the absorbance was recorded at 228 nm, and the chromatogram was integrated using the Agilent Chemstation (Agilent Technologies) enhanced integrator for detection of liberated hippuric acid (A). Hippuric acid standard

was used to calibrate the standard curve and retention time. The percentage inhibition was calculated by:

$$\% \text{ inhibition } = \frac{(A_{\text{control}} - A_{\text{extract}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100$$

4.1.4.8 HPLC Analysis of Phenolic Phytochemicals

Two milliliters of the extracts was filtered (pore size, 0.2 μm), and 5 μL was injected in the HPLC apparatus (Agilent 1100 series equipped with autosampler and DAD 1100 diode array detector). The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% over the next 7 minutes, then decreased to 0% for the next 3 minutes, and maintained for the next 7 minutes (total run time, 25 minutes). The analytical column used was an Agilent Zorbax SB-C18, 250 mm × 4.6 mm i.d., with packing material of 5 μm particle size at a flow rate of 1 mL/minute at ambient temperature. During each run the absorbance was recorded at 306 nm and 333 nm, and the chromatogram was integrated using the Agilent Chemstation enhanced integrator. Calibration was performed by injecting the standards of cinnamic acid, rosmarinic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, and quercetin. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards. The results were expressed as μg/g of sample DW.

4.1.4.9 Statistical Analysis

All experiments were performed in either duplicates or triplicates. Analysis at every time point from each experiment was carried out in duplicate or triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP.

4.1.5 Results and Discussion

4.1.5.1 Total Soluble Phenolics and Antioxidant Activity

Potato has known to be domesticated in the Andes of Southern Peru about 10,000 years ago (Ames and Spooner, 2008). The type of potato grown in Chile (*Solanum tuberosum ssp. tuberosum L.*) is known to be one of the highest yielding, along with potatoes from the region of Argentina and Venezuela (Ames and Spooner, 2008). Not many studies have been carried out to explore the health benefits of Chilean potato (*Solanum tuberosum ssp. tuberosum L.),* in the context of its relevance for managing of type II diabetes and hypertension through the diet. Although, it is known that the consumption of potato with higher nutritional and bioactive profiles has the potential to manage chronic diseases, its consumption in processed and fried forms such as French fries and potato chips has raised health concerns. Such high fat processed forms potentially increase risks for obesity-linked chronic diseases due to very high calorie intake from higher than normal servings.

The amounts of total phenolic content between the 54 different Chilean potato (*Solanum tuberosum ssp. tuberosum L.)* cultivars (varieties) were evaluated. The different colors of potato samples indicated that, bright color potato samples potentially have higher antioxidant and phenolics activity. Anthocyanins have been known to have biological functions such as high antioxidant, antimicrobial and anti-obesity potential (Zhang *et al*., 2009). It has been reported that, purple color of potato seems to be an indication of anthocyanin presence, which is 3-4 times higher in the potato peel compared

to tuber (Zhang *et al*., 2009). The variations ranged with high total phenolic content for dark colors (purple, red and pink) and low phenolic content for light colors (white and yellow) potatoes. Therefore we can assume that different peel colors of potato affects the activity of antioxidants in a particular sample. The sample extraction method utilized was the same for all 54 samples of Chilean potato (*Solanum tuberosum ssp. tuberosum L.),* where 2.5 g of sample was extracted in 100 mL of water at 95°C for 30 minutes. Based on these studies, enhancement and development of potato in accordance to increasing their phenolic content would prove to be beneficial as a diet based management of oxidation-linked chronic diseases such as type II diabetes.

Therefore, in this study, we focused on aqueous extracts of 54 different samples of Chilean potato (*Solanum tuberosum ssp. tuberosum L.),* which were further evaluated for hyperglycemia-linked anti-diabetic potentials and also for complications such as hypertension using the appropriate *in vitro* assays as rapid screening tool. Figures 1, 2 and 3 illustrate the results for total phenolic content and total antioxidant activity of aqueous extracts for all samples. The total phenolic content of Chilean potatoes ranged from 0.92 mg/g (PA 43) to 13.1 (PA 4) mg/g of sample dry weight (DW). The total phenolic content of all these samples showed a high correlation ($r = 0.83$) with antioxidant activity by DPPH assay. Observing the sample colors indicated that high phenolic and antioxidant activities were related with darker colors compared to low activities linked to white and yellow varieties. In this study, many potato samples consisted of high phenolic and antioxidant activity in aqueous extracts, which indicates its potential for a good source of phenolic antioxidants.

The most important vitamin obtained from fruits and vegetables for human health and nutrition is vitamin C. Potato is rich in vitamin C with the content of 15 mg/100 g of steamed potato (Robert *et al*., 2006). The amount of vitamin C in a potato contributes to 25-30% of our recommended dietary allowance (Robert *et al*., 2006). The high amount of overall antioxidants from both phenolics and vitamin C in potatoes can support our bodies against oxidative complications linked to type II diabetes and cardiovascular diseases, by limiting oxidative stress (Chu *et al*., 2002; Robert *et al*., 2006). Figures 1, 2 and 3 show the results for overall antioxidant activity by DPPH assay for 54 different Chilean potato varieties. The *in vitro* antioxidant potential measured via DPPH inhibition assay for potato samples ranged from 5.8% (PA 29) to 77% (PA 21). We can assume that, Chilean potatoes with high antioxidant activity can be beneficial towards limiting the oxidative stress and helping to manage the micro vascular oxidative stress-linked complications of hyperglycemia and hypertension. High positive correlation was found between total phenolics and antioxidant activity through DPPH assay for aqueous extracts ($r = 0.83$).

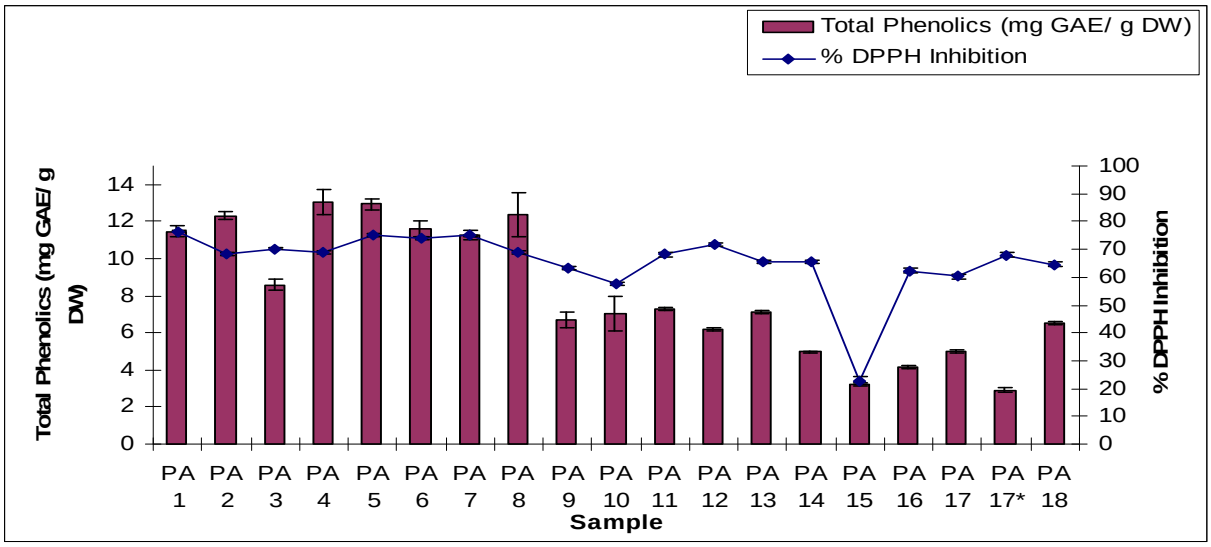

Figure 1 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 1-18.

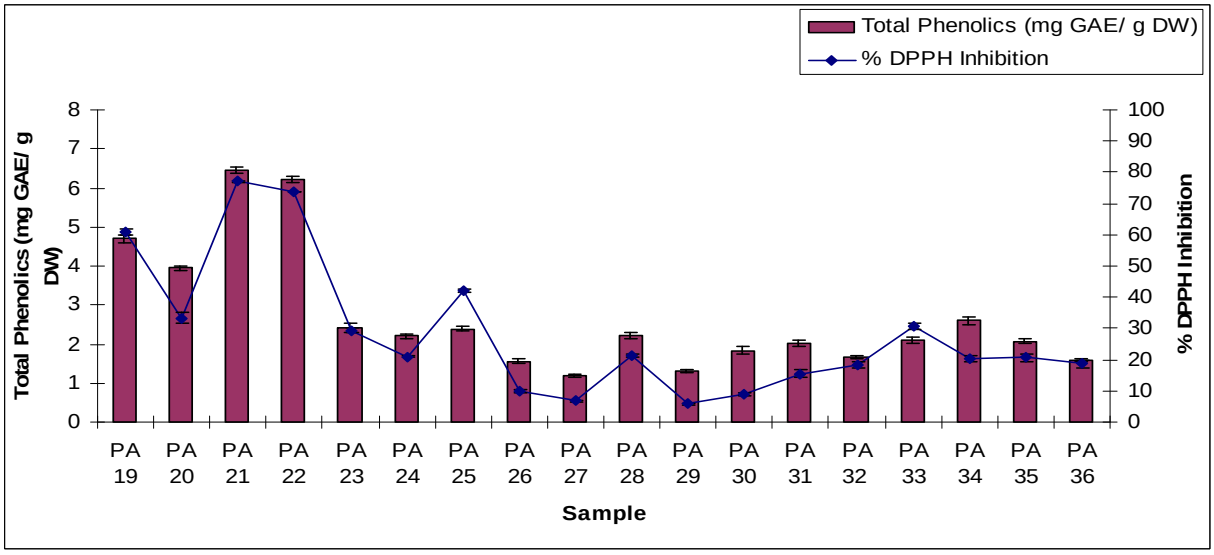

Figure 2 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 19-36.

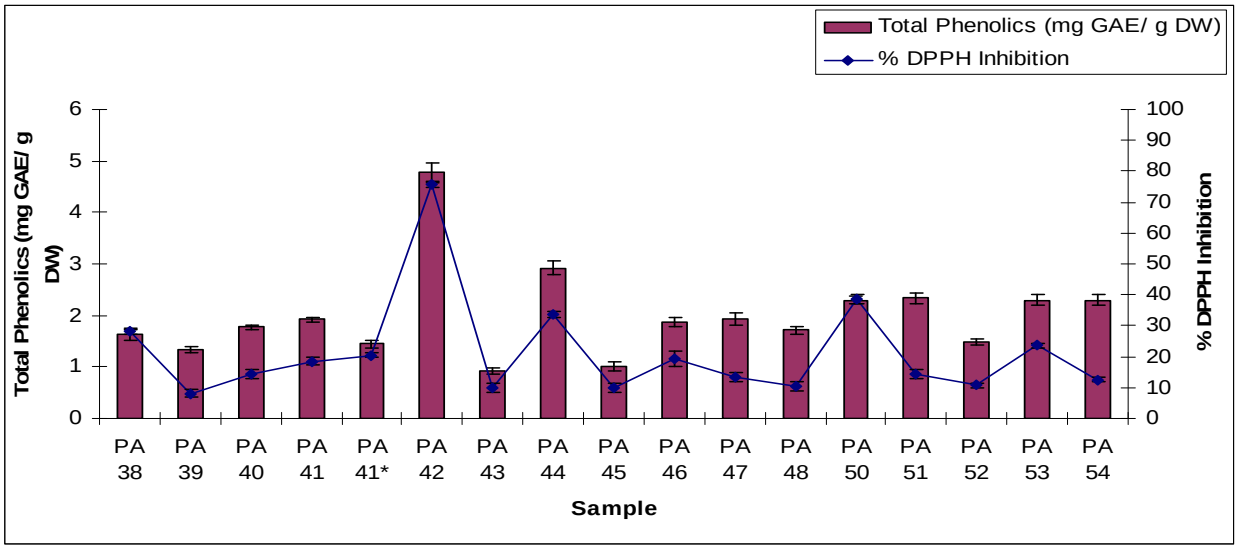

Figure 3 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 38-54.

4.1.5.2 α–Glucosidase Inhibition Assay

In this study, we investigated health benefits of Chilean potato (*Solanum tuberosum ssp. tuberosum L.)* for its hyperglycemic management potential linked to type II diabetes. According to World Health Organization, 90% of people suffering from diabetes mellitus around the world have type II diabetes (World Health Organization, www.who.org). Type II diabetes is characterized through a constant increase in blood glucose levels due to breakdown of starch foods by α- amylase and absorption of glucose in small intestine by α–glucosidase (Ranilla *et al*., 2010). α–Glucosidase inhibitory enzyme contributes in management of hyperglycemia, linked to type II diabetes (Pinto *et al*., 2008; Shetty et al., 2010). Therefore early stages of type II diabetes could be controlled via inhibition of α–glucosidase, which participates in the overall digestion and

uptake of carbohydrates from the diet. Varieties that indicate high α–glucosidase inhibitory activities can be further used *in vivo* studies as part of therapeutic or clinical strategy, for managing hyperglycemia linked to type II diabetes.

Dose dependency (10 µL, 25µL, 50µL) of all samples evidently indicated dose dependent response. Figures 4, 5 and 6 illustrate results of α–glucosidase inhibition for all samples. The *in vitro* α–glucosidase inhibitory activity ranged from 0% (PA 17*, 35, 36, 38, 39, 40, 41, 52, 53, 54) to 59% (PA 13), and a large scale of variation was seen between all 54 varieties of Chilean potato (Figure 4-6).

Extraction method for sample preparation was the same for α–glucosidase inhibition assay, which is described above. Samples with no dilution (50 µL), indicated the highest inhibition. Moderate correlation was observed between α–glucosidase inhibition and total phenolic content for aqueous extracts of all samples ($r = 0.50$). Also, a moderate correlation was seen between antioxidant activity through DPPH assay and α–glucosidase inhibition ($r = 0.54$). We can therefore suggest that total phenolic content and free radical-linked antioxidant activity is associated with α–glucosidase inhibitory activity, since a moderately good correlation was observed. Also, these correlations may suggest that the highest inhibition of α–glucosidase could be due to darker colors of potato skins compared to lighter ones. Therefore, inhibitors in various skin samples of Chilean potato can be utilized in therapeutic strategies to manage hyperglycemia. These therapies and clinical strategies should be applied in early stages to manage hyperglycemia for the prevention of type II diabetes.

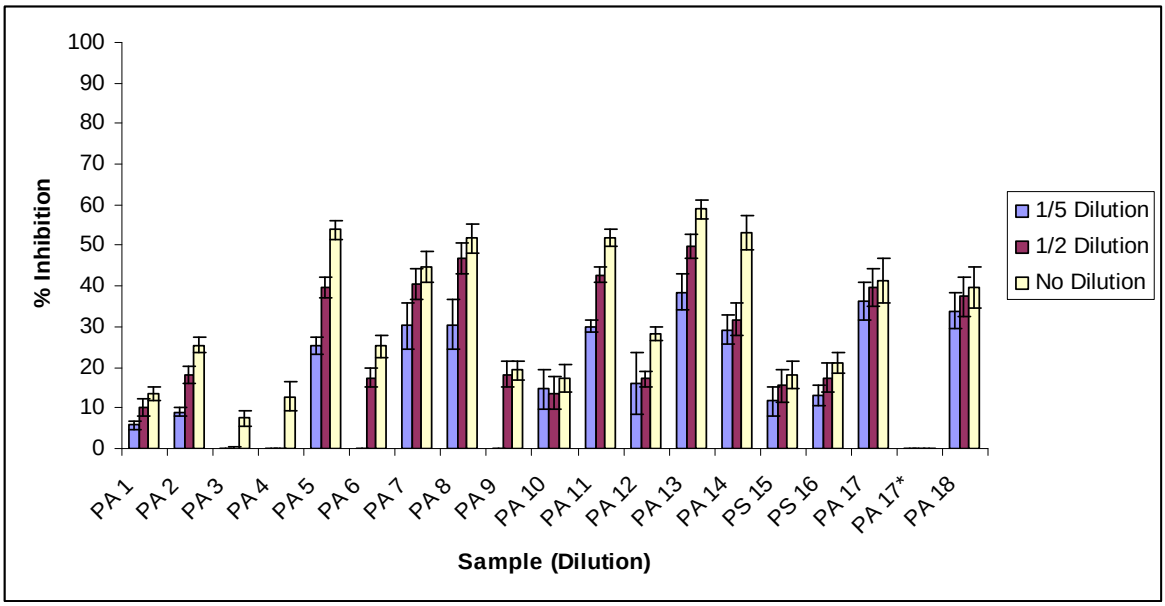

Figure 4 Changes observed in dose dependent (10 µL, 25 µL and 50 µL) percent α-glucosidase inhibitory activity of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 1-18.

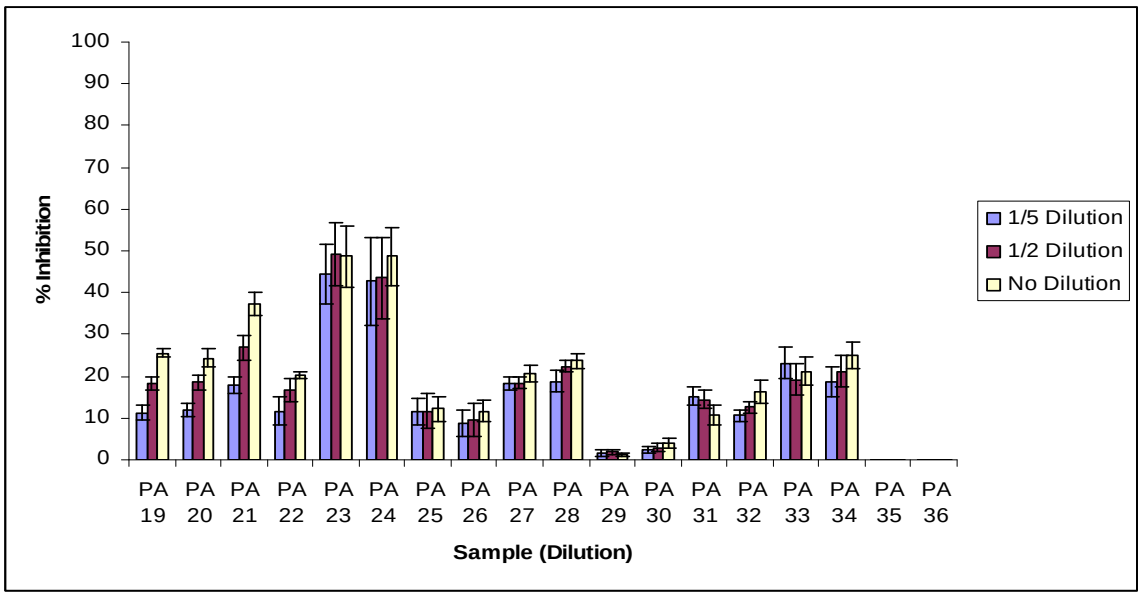

Figure 5 Changes observed in dose dependent (10 µL, 25 µL and 50 µL) percent α-glucosidase inhibitory activity of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 19-36.

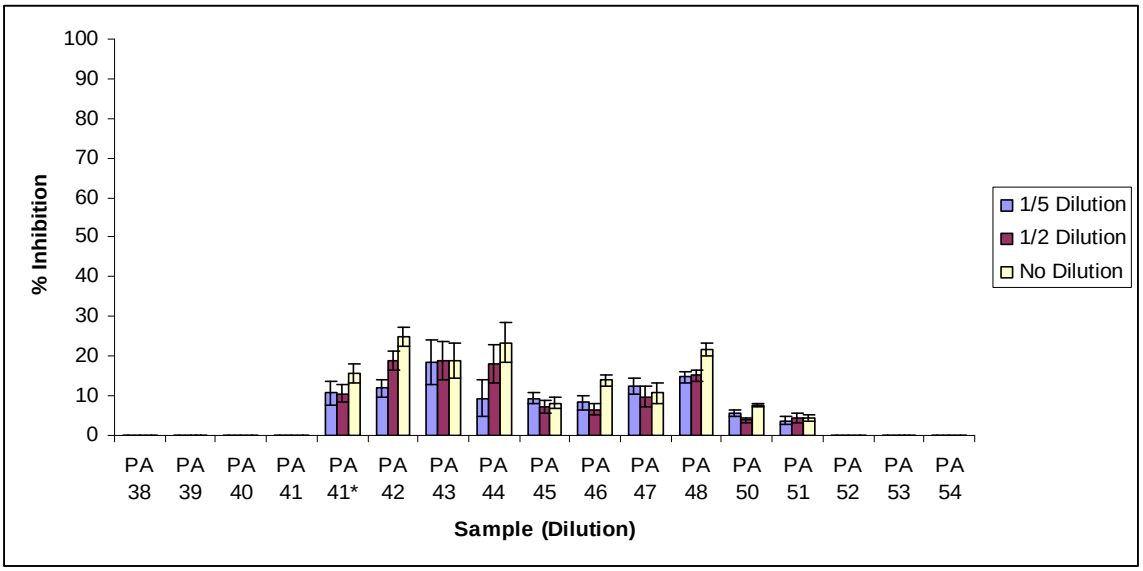

Figure 6 Changes observed in dose dependent (10 µL, 25 µL and 50 µL) percent α-glucosidase inhibitory activity of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 38-54.

4.1.5.3 α- Amylase Inhibition Assay

α-Amylase is another enzyme similar to α–glucosidase, which is known to have an important role in managing hyperglycemia linked to type II diabetes, though strong inhibition could lead to undigested starch-linked side-effects (Kwon *et al*., 2006; Pinto *et al*., 2009). *In vitro* α-amylase inhibition was evaluated for all 54 Chilean potato (*Solanum tuberosum ssp. tuberosum L.)* varieties, with the same protocol for aqueous sample preparation as rest of the inhibition assays described above. There was no α-amylase inhibition observed for any of the samples (PA 1-54). It has been suggested that α-amylase inhibitory activity could possibly be related to some specific phenolic compounds (Pinto *et al*., 2009), which might not be present in samples observed in this study. Though α-amylase inhibitory activity is low the benefits of α–glucosidase

inhibition is reasonable for select varieties to be considered as a component of a whole food design that can be part of our diet to help manage hyperglycemia linked to type II diabetes in its early stages (Pinto *et al*., 2009; Shetty *et al*., 2010).

4.1.5.4 ACE Inhibition Assay

Cardiovascular diseases (CVD) are known to be one of the leading causes of deaths worldwide. Diets rich in fruits and vegetables are recommended for prevention of CVD, and its consumption lowers the risk of CVD including hypertension, which is a macro vascular complication of type II diabetes (Bazzano *et al*., 2003; Kwon et al., 2006). Fruits and vegetables include combinations of micronutrients, antioxidants, phytochemicals and fibers which may be able to reduce the risk of cardiovascular diseases (Liu *et al*., 2000). ACE inhibition is an important target for hypertension management, and in this study we have evaluated inhibition of this particular enzyme in response to aqueous potato extracts. The evaluation of 54 different varieties of Chilean potato (*Solanum tuberosum ssp. tuberosum L.)* with varying colors, would also give us an idea of how important total phenolic content is in the context of anti-hypertensive potentials of a specific varieties.

Figure 7, 8 and 9 shows ACE inhibitory activity for aqueous extracts of all potato samples. The *in vitro* ACE inhibitory activity ranged from 0% (PA 2, 9, 14, 15, 20, 22-27, 29-31, 33, 40, 48-51) to as high as 88% (PA 17). ACE inhibitory activity showed high variance for its inhibitions. Low correlation was observed between total phenolics and ACE inhibition ($r = 0.13$). Also, a low correlation was observed between antioxidant

activity by DPPH assay and ACE inhibition ($r = 0.21$). Correlations observed in this study suggest that ACE inhibitory activity does not depend on specific phenolic compounds present in potato samples. .Same observation can be true for antioxidant activity, because the correlation does not show relevant activity between aqueous extracts of DPPH assay and ACE inhibition. Specific phenolic compounds found in potato may be able to reduce the risk of hypertension, type II diabetes and other various conditions for cardiovascular diseases. It is also possible that bioactive components other than phenolics may be responsible for ACE inhibitory activity (Kwon *et al*., 2006; Pinto *et al*., 2009).

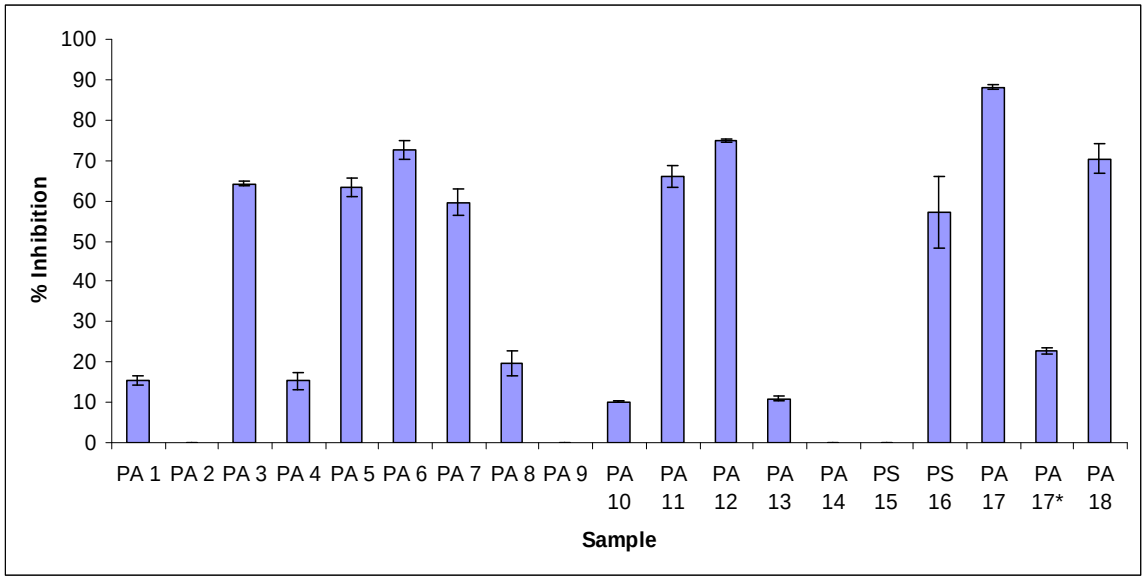

Figure 7 ACE inhibitory activity (% Inhibition) of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 1-18.

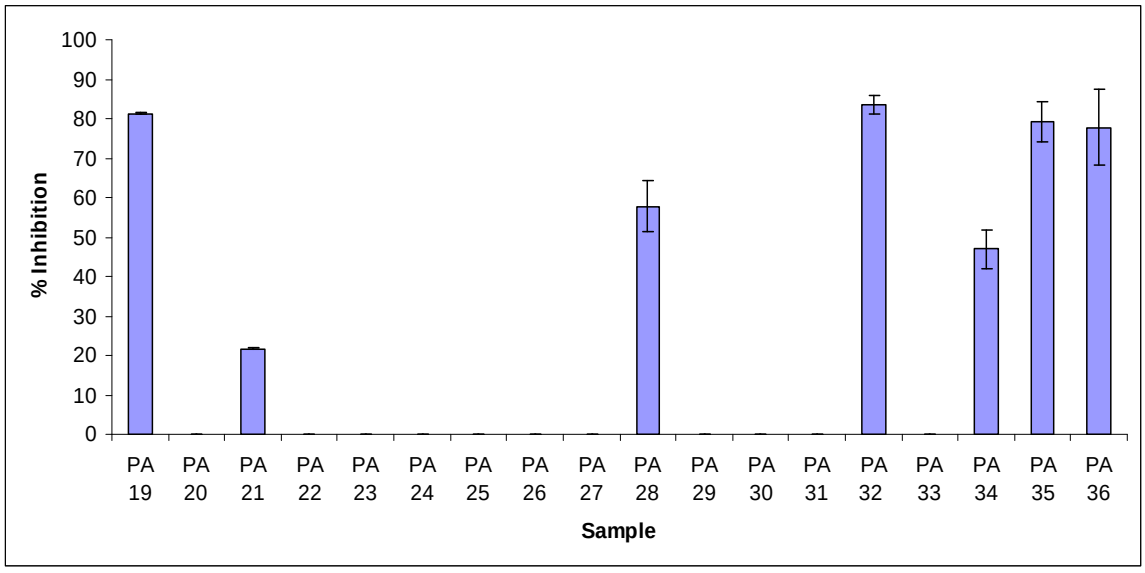

Figure 8 ACE inhibitory activity (% Inhibition) of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 19-36.

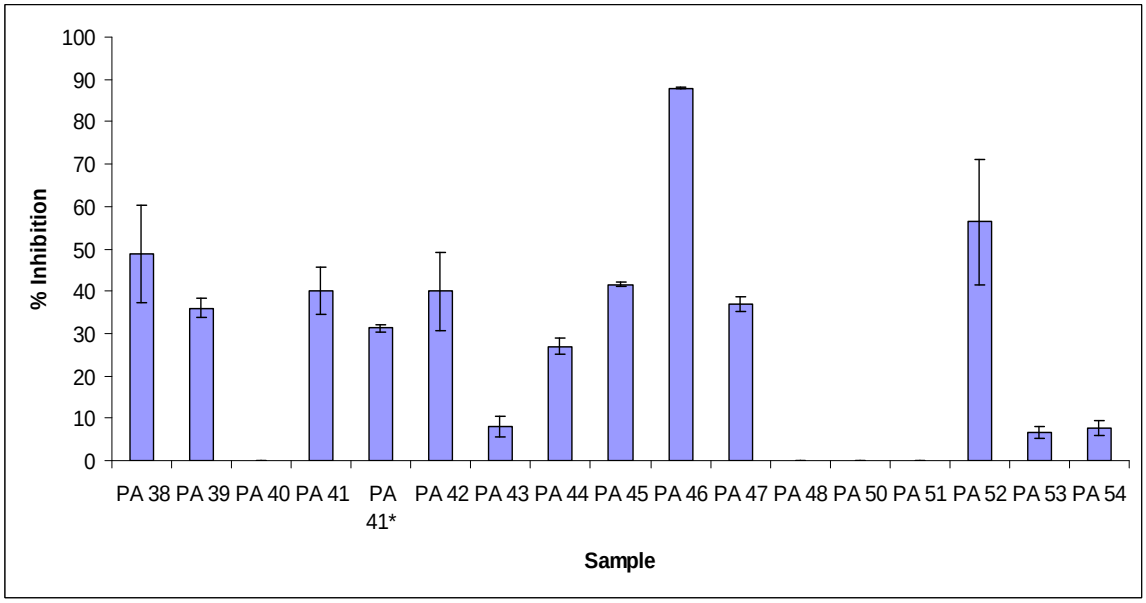

Figure 9 ACE inhibitory activity (% Inhibition) of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) for samples PA 38-54.

4.1.5.5 HPLC Analysis of Phenolic Phytochemicals

Regular intake of natural phenolic compounds through our diet has beneficial affects on our overall health through potential reduction of oxidation-linked chronic diseases. Aqueous extracts of all samples were analyzed for phenolic profile via HPLC. Table 1, 2 and 3 includes data for different compounds found in potatoes. The peaks were identified based on retention time and ultraviolet absorption spectra of corresponding standards (Pinto *et al*., 2009). The five phenolic compounds found in the samples included chlorogenic acid, ferulic acid, caffeic acid, p-coumaric acid and catechin.

Chlorogenic acid is known to contain about 90% of total phenolic compounds of potato tubers (Dao and Friedman, 1992), and might play an important role in quality and safety of potato plant. Phenolic profile analysis of chlorogenic acid ranged from absence (PA 27, 30, 34, 39, 41*, 48) to 17.9 mg/g (PA 8) of sample DW. Chlorogenic acid has the ability to lower blood pressure in mildly hypertensive patients, and its derivatives have shown to lower blood pressure in hypertensive rats (Cheplick *et al*., 2010). The phenolic compound ferulic acid analyzed in potatoes ranged from absence in some samples (PA 27, 29, 30, 31, 36, 39, 40, 43, 48, and 52) to 10.5 mg/g (PA 21) of sample DW. Ferulic acid has a potential via natural sources to prevent cardiovascular disease and diabetes (Zhao *et al*., 2008). Potato along with eggplant and tomato are good sources of ferulic acid, which are shown to supply anywhere from 5-70 mg of ferulic acid/ 100 g (Zhao *et al*., 2008). Caffeic acid was observed to be absent (PA 1, 3, 11, 17*, 36, 51 and 54) to 10.3 mg/g (PA 42) of sample DW. Caffeic acid has been observed to have beneficial potential for managing cardiovascular diseases, as well as its hypotensive affects in

hypertensive rats (Li *et al*., 2005). Another phenolic compound observed in potato samples included p-coumaric acid, which ranged from absent (PA 15, 24-33, 36, 40, 45, 48 and 54) to 7.3 mg/g (PA 21) of sample DW. The last phenolic compound observed was catechin, which ranged from absence 10, 15, 18, 22) to 4.35 mg/g (PA 54) of sample DW. Matsui *et al.* (2007) has suggested that catechin has the potential to prevent diabetes through natural intake in our diet. Catechins have heart diseases and cancer prevention potential, but another study (Nagao *et al*., 2007) was carried out to observe its fat reducing abilities. Continuous consumption of catechins via our diet, especially in high amounts, indicated reduction of fat, cholesterol levels and blood pressure without drastic changes in our lifestyles (Nagoa *et al*., 2007). Therefore, we can assume that inclusion of the right sub-tropical cultivars of Chilean potato could be potentially beneficial towards our health with potential for prevention of hyperglycemia and hypertension in early stages of type II diabetes and associated cardiovascular diseases in the long term.

Table 1 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Chilean Potato (*Solanum tuberosum ssp. tuberosum L.*): Samples 1-18.

SAMPLE	Chlorogenic Acid	Ferulic Acid	Caffeic Acid	P-Coumaric Acid	Catechin
	mg/g DW	mg/g DW	mg/g DW	mg/g DW	mg/g DW
PA 1	13.9 ± 2.99	0.23 ± .003		3.08 ± 0.17	3.08 ± 2.17
PA 2	14.9 ± 3.72	4.32 ± 0.51	6.77 ± 2.73	0.13 ± .003	0.13 ± 0.09
PA 3	11.7 ± 3.36	1.18 ± 0.31		1.05 ± 0.40	0.62 ± 0.44
PA 4	16.5 ± 6.38	1.79 ± 0.39	2.58 ± 0.02	2.16 ± 0.32	1.61 ± 1.14
PA 5	15.9 ± 4.42	2.27 ± 0.26	1.59 ± 0.05	0.57 ± 0.22	0.96 ± 0.68
PA 6	13.3 ± 4.17	1.35 ± 0.39	1.12 ± 0.02	0.50 ± 0.10	0.64 ± 0.45
PA 7	16.2 ± 4.98	0.82 ± 0.27	0.94 ± 0.03	1.87 ± 0.59	1.21 ± 0.85
PA 8	17.9 ± 5.59	2.20 ± 1.14	0.35 ± .004	1.19 ± 0.54	0.21 ± 0.10
PA 9	3.50 ± 0.72	1.51 ± 0.50	0.20 ± 0.04	0.12 ± .001	2.49 ± 1.76
PA 10	2.39 ± 1.16	1.09 ± 0.57	1.85 ± 0.49	1.36 ± .001	
PA 11	19.3 ± 5.12	6.27 ± .006		2.23 ± 0.87	0.71 ± 0.50
PA 12	15.6 ± 4.29	1.30 ± 0.30	1.34 ± 0.12	1.19 ± 0.18	0.89 ± 0.63
PA 13	15.5 ± 2.76	4.26 ± 1.42	3.24 ± 0.01	1.82 ± 0.60	0.77 ± 0.55
PA 14	8.92 ± 2.26	1.81 ± 0.62	1.15 ± 0.20	2.22 ± 0.44	1.44 ± 1.02
PA 15	4.82 ± 1.33	0.47 ± 0.16	0.83 ± .001		
PA 16	6.86 ± 1.93	1.44 ± 0.26	0.60 ± .001	0.55 ± .007	0.74 ± 0.53
PA 17	5.95 ± 1.60	1.11 ± 0.36	0.92 ± 0.21	1.40 ± 0.26	0.94 ± 0.66
PA 17*	6.36 ± 1.39	0.53 ± 0.19		1.37 ± 0.41	1.56 ± 0.78
PA 18	13.3 ± 2.11	2.28 ± 0.69	1.33 ± 0.46	4.66 ± 0.06	

Table 2 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Chilean Potato (*Solanum tuberosum ssp. tuberosum L.*): Samples 19-36.

SAMPLE	Chlorogenic Acid	Ferulic Acid	Caffeic Acid	P-Coumaric Acid	Catechin
	mg/g DW	mg/g DW	mg/g DW	mg/g DW	mg/g DW
PA 19	6.44 ± 2.51	2.08 ± 0.45	0.91 ± 0.07	1.58 ± 0.41	0.22 ± 0.07
PA 20	7.23 ± 2.60	1.05 ± 0.38	0.15 ± 0.07	0.36 ± 0.27	0.22 ± 0.08
PA 21	0.15 ± 0.00	10.5 ± 1.34	3.99 ± 0.74	7.31 ± 2.66	0.43 ± .006
PA 22	19.6 ± 7.68	3.39 ± 0.19	0.72 ± .004	3.06 ± 1.13	
PA 23	2.11 ± 0.24	0.05 ± 0.01	1.54 ± 0.55	0.14 ± 0.05	0.11 ± 0.04
PA 24	1.99 ± .002	0.10 ± 0.02	0.86 ± 0.25		0.22 ± 0.09
PA 25	6.42 ± 2.26	0.71 ± 0.27	1.53 ± 0.54		0.53 ± 0.13
PA 26	0.09 ± .001	0.15 ± .001	0.16 ± .002		0.54 ± 0.12
PA 27			0.10 ± 0.01		0.60 ± 0.12
PA 28	0.21 ± 0.05	0.03 ± .002	0.49 ± .003		1.06 ± 0.14
PA 29	0.16 ± .001		0.06 ± 0.00		0.80 ± 0.27
PA 30			0.81 ± 0.19		0.16 ± 0.05
PA 31	0.07 ± .005		0.19 ± 0.02		1.92 ± 0.57
PA 32	0.44 ± 0.02	0.16 ± 0.07	2.54 ± 0.03		0.64 ± 0.11
PA 33	1.53 ± 0.41	0.33 ± 0.07	3.02 ± 0.25		1.71 ± 0.04
PA 34		0.15 ± 0.06	3.28 ± 0.68	0.18 ± 0.03	2.17 ± 0.16
PA 35	0.47 ± 0.06	0.18 ± .005	0.51 ± .006	0.07 ± .007	1.05 ± 0.03
PA 36	3.00 ± 0.54				0.42 ± .002

Table 3 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Chilean Potato (*Solanum tuberosum ssp. tuberosum L.*): Samples 38-54.

SAMPLE	Chlorogenic Acid	Ferulic Acid	Caffeic Acid	P-Coumaric Acid	Catechin
	mg/g DW	mg/g DW	mg/g DW	mg/g DW	mg/g DW
PA 38	1.54 ± 0.49	0.47 ± 0.04	0.95 ± 0.29	0.13 ± .002	0.34 ± 0.18
PA 39			0.07 ± 0.01	0.04 ± .005	1.03 ± 0.16
PA 40	1.94 ± 0.01		0.88 ± 0.23		1.45 ± 0.36
PA 41	3.22 ± 0.06	0.03 ± 0.00	2.07 ± .009	0.05 ± 0.01	1.31 ± 0.45
PA 41*		0.29 ± 0.11	3.28 ± 0.51	0.04 ± 0.10	1.62 ± 0.48
PA 42	4.76 ± 1.72	0.86 ± 0.22	10.3 ± 0.11	1.00 ± 0.25	2.73 ± 0.11
PA 43	1.41 ± 0.48		0.41 ± 0.08	0.03 ± .001	0.87 ± 0.26
PA 44	1.14 ± 0.29	0.04 ± .007	0.86 ± 0.24	0.66 ± 0.06	2.31 ± 0.20
PA 45	0.25 ± .003	0.09 ± 0.02	0.80 ± 0.19		1.04 ± 0.24
PA 46	1.33 ± 0.27	0.14 ± 0.06	4.04 ± 0.83	0.68 ± 0.11	1.55 ± 0.16
PA 47	0.28 ± .002	0.06 ± 0.01	0.28 ± 0.04	0.13 ± 0.02	1.32 ± 0.16
PA 48			0.14 ± .006		2.26 ± 0.97
PA 50	2.46 ± 0.01	0.08 ± .006	2.33 ± 1.06	0.17 ± .002	1.33 ± 0.13
PA 51	0.61 ± 0.08	0.04 ± .003		0.05 ± .002	4.15 ± 0.84
PA 52	1.01 ± 0.53		1.58 ± .022	0.03 ± .001	0.98 ± 0.46
PA 53	0.98 ± .002	0.07 ± .007	0.46 ± 0.02	0.05 ± .005	2.64 ± 0.57
PA 54	0.53 ± 0.03	0.03 ± .001			4.35 ± 0.72

4.1.6 Conclusions

In this study, we have evaluated anti-diabetic and anti-hypertensive management potential of Chilean Potatoes (*Solanum tuberosum ssp. tuberosum L.*) linked to type II diabetes using *in vitro* models. We utilized the usual preparation method of potato through hot water extraction. Total soluble phenolics, total antioxidant activity by DPPH inhibition and functionality assays such as α-glucosidase inhibition, α-amylase inhibition and ACE inhibition were also evaluated. The results of this study indicated that Chilean potatoes do not have α-amylase inhibitory potential. However, the. α-glucosidase inhibition observed show a large variation between all 54 samples. The inhibition ranged upto 60%, which indicates that some potato varieties could be considered part of our diet to prevent hyperglycemia linked to type II diabetes. Some potato varieties observed showed a high ACE inhibition activity, which indicated that specific varieties could be potentially beneficial towards prevention of hypertension. This study provides important information on functions of several Chilean potato varieties, so appropriate varieties could be part of breeding programs and food designs to be part of diets to prevent hyperglycemia and hypertension linked to type II diabetes. Based on wide screening using *in vitro* assays and a strong biochemical rationale, specific varieties could be part of diet designs for animal and clinical studies.

4.2 Anti-diabetic Potential and Seed Phytochemicals of Select Species of Family Apiaceae Using *In Vitro* Assays

4.2.1 Abstract

A global epidemic of type II diabetes worsens food-based therapeutic strategies for the prevention and management is being explored. Water and 12% ethanol extracts of seeds of six select species of family Apiaceae were evaluated for total soluble phenolic assay and DPPH inhibition assay. *In vitro* assays such as α-glucosidase and α-amylase inhibition for anti-hyperglycemia potential and angiotensin I-converting enzyme (ACE) inhibition for anti-hypertension potential relevant to type II diabetes management were evaluated. Total phenolic content for aqueous extracts ranged from 4.8 mg/g (Coriander) to 8.3 mg/g (Ajowan) of DW. Total phenolic content and antioxidant activity for aqueous extracts indicated a moderate correlation ($r = 0.61$), and a high correlation ($r = 0.73$) for ethanolic extracts. α-Glucosidase inhibitory activity for aqueous extracts ranged from 16.5% (Caraway) to 50% (Dill). These *in vitro* assays indicate that select family Apiaceae species used as food condiments have anti-diabetic potentials. This provides the biochemical basis for therapeutic strategies for including these condiments as part of improved diet for prevention of hyperglycemia and associated hypertension.

4.2.2 Industrial Relevance

Utilizing plant sources as medicinal food has been widely used throughout the world since ancient times. Developing strategies to explore efficacy of plant sources towards prevention of hyperglycemia and hypertension linked to type II diabetes would serve as the basis for innovation towards industrial applications. This study investigates functionalities of seed extracts of select species from family Apiaceae with potential for therapeutic and medicinal uses for prevention of hyperglycemia and hypertension linked to type II diabetes. This strategy has low toxicity compared to synthetic drugs. Also, availability of plant sources in localized communities based on local bioresources for disease management is potentially easier and less expensive than synthetic medicines. Therefore understanding functionalities of seed sources of family Apiaceae would lead to dietary strategies for hyperglycemia and hypertension management, which would be available as condiments at a low cost to wider communities globally.

4.2.3 Introduction

Type II diabetes results from the inability of our body's to response to high blood glucose levels from high carbohydrate diets leading to several metabolic and pathological changes. Type II diabetes is one of the most common diseases affecting the people of both developed and developing countries, affecting about 5%-7% of the population in several countries. The number of people suffering from type II diabetes is believed to be rising constantly worldwide and is projected to increase to 400 million by 2030. Plant-based medicinal drugs have been used for various disease therapies since ancient times. Even though there has been a considerable progress in managing type II diabetes through synthetic drugs, investigation for natural anti-diabetic plant and food products for its management has potential to offer effective less expensive strategies. In addition hypoglycemic activities of many plant species have been discovered, acting as anti-metabolites to help block the oxidation pathway of fatty acids (Ahmad *et al*., 2009).

Plants from family Apiaceae are commonly used as food, flavoring of foods and for their medicinal purposes. Seeds from family Apiaceae have been used as a common household remedy for many complications such as hypertension (Gilani *et al*., 2005). Plants from Apiaceae family are used in Rajasthan, India to treat common ailments such as stomachaches, abdominal pain and acidity (Shekhawat and Batra, 2006). Mixture of different plants is consumed orally with water or applied externally through massaging abdomen with it (Shekhawat and Batra, 2006). In parts of the world such as Cameroon, plants from Apiaceae family are used as therapies for vomiting, appendicitis, ingestion, constipation and treating mosquito bites (Jiofack *et al*., 2009).

Ancient form of Indian medicine 'Ayurveda' uses the active ingredients from plants for treatment of various diseases (Dhandapani *et al*., 2002). Compared to synthetic drugs, plant products are less toxic and have less of side effects. Plants from Apiaceae family are frequently considered to have hypoglycemic affects on humans and animals (Dhandapani *et al*., 2002). Hypoglycemic action of plants are due to insulin effect by increasing pancreatic secretion of insulin from cells of islets of Langerhan's or its release from bound insulin (Dhandapani *et al*., 2002).

Additionally other seed extracts from Apiaceae family have been observed for their health beneficial effects. Essential oils from *Foeniculum vulgare* seeds (Fennel) such as anethole and limonene are used for medicinal purposes and their seeds are also used as tranquillizers and tonics (Oktay *et al*., 2003). Aqueous extracts of fennel are observed for its hypotensive effect in a dose-related manner (Oktay *et al*., 2003). *Anethum graveolens* seed (Dill) extracts are used to treat diarrhea and scours as well as feeding it to goats and cows for enhancing milk production (Lans *et al*., 2007). *Coriandrum sativum* seeds (Coriander) are mainly used as food flavoring agent and also used to treat ulcers (Husain *et al*., 2008). *Trachyspermum copticum* (Ajowan) seeds are frequently used to treat diarrhea, ingestion and common colds. Ajowan seed oils are suggested to treat medical problems affecting joints or painful muscle conditions (Husain *et al*., 2008). *Carum carvi* (Caraway) is suggested to have beneficial effects for treatment and management of diabetes and cardiovascular diseases. It evokes beneficial effects on the elevation of lipids in the bloodstream associated with hyperglycemia (Lemhadri *et al*., 2006). *Pimpinella anisum* (Anise) is a plant belonging to Apiaceae family native to the Middle East which is used as aromatic spice and to help digestion (Arslan *et al*., 2004).

Understanding the biochemical basis for medicinal uses and phytotherapies of Apiaceae family with a focus on their seeds would provide us with a basis for further clinical research which will provide insight on how they could be targeted towards prevention of certain chronic diseases. Plant-based medicinal treatments for targeted prevention of diabetes and other diseases are going to play an important part due to its low toxicity potentials and low cost for wide usage. In this study, we have investigated functionalities of seeds of 6 select species of family Apiaceae, and how their *in vitro* effects could provide a biochemical rationale to potentially target them towards the prevention and management of hyperglycemia and hypertension linked to type II diabetes. We evaluated total soluble phenolics and DPPH inhibition assay to determine total antioxidant activity and used High Performance Liquid Chromatography for analysis of phenolic phytochemicals of seeds. *In vitro* assays such as α-glucosidase, α-amylase and angiotensin converting enzyme (ACE) inhibitory activities were performed to evaluate seeds potential towards hyperglycemia and hypertension management as part of dietary strategies for management of type II diabetes and its complications.

4.2.4 Materials and Methods

4.2.4.1 Materials

Apiaceae family seeds were Swad brand dill, ajowan, fennel, coriander and anise were purchased from a local Asian grocery store (Asian-American International) in Hadley, MA, USA. Caraway sample evaluated was purchased from Whole Foods Market Hadley, MA, USA. Porcine pancreatic α-amylase (EC 3.2.1.1), rat intestinal α-glucosidase (EC 3.2.1.20), hippuric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), rabbit lung ACE (EC 3.4.15.1), cinnamic acid, rosmarinic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO).

4.2.4.2 Sample Preparation

Water Extracts:

The samples (2.5 g) were extracted in 100 mL of distilled water under reflux at 95°C for 30 minutes. The samples were centrifuged for 10 minutes.

Ethanol extracts:

The samples (2.5 g) were extracted in 100 mL of 12% ethanol in a shaker at a speed of 150 RPM overnight at 20°C. The samples were filtered and stored in a refrigerator at –20°C until analysis, for no more than 1 week.

4.2.4.3 Total Soluble Phenolic Assay

The total phenolics in all samples were determined by using a method modified by Shetty *et al*. (1995). In brief; 0.5 ml of sample extract was added to a test tube and mixed with 0.5 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (vol/vol) Folin-Ciocalteu reagent was added and mixed. The absorbance was read at 725 nm using a spectrophotometer (Genesys UV/Visible, Milton Roy, Inc., Rochester, NY). Different concentrations of gallic acid were used to develop a standard curve. Results were expressed as mg of gallic acid/g of sample dry weight (DW).

4.2.4.4 Total Antioxidant activity by DPPH Radical Inhibition Assay

The antioxidant activity was determined by the DPPH radical scavenging method modified from Kwon *et al*. (2006). A 250-μL aliquot of the sample extract was mixed with 1,250 μL of DPPH (60 μ*M* in ethanol). The mixture was centrifuged at 13,000 *g* for 1 minute, and after this the absorbance was measured at 517 nm using the Genesys UV/Visible spectrophotometer. The readings were compared with the controls, containing 95% ethanol instead of sample extract. The percentage inhibition was calculated by:

$$\%\ \text{inhibition} = \frac{(Absorbance_{\text{control}} - Absorbance_{\text{extract}})}{Absorbance_{\text{control}}} \times 100$$

4.2.4.5 α-Amylase Inhibition Assay

The α-amylase inhibitory activity was determined by an assay modified from the *Worthington Enzyme Manual (*Worthington, 1993). A total of 500 µL of sample extract and 500 µL of 0.02 *M* sodium phosphate buffer (pH 6.9 with 0.006 *M* NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25°C for 10 minutes. After preincubation, 500 µL of a 1% starch solution in 0.02 *M* sodium phosphate buffer (pH 6.9 with 0.006 *M* NaCl) was added to each tube at timed intervals. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted after adding 5-15 mL of distilled water, and the absorbance was measured at 540 nm using the Genesys UV/Visible spectrophotometer. The readings were compared with the controls, containing buffer instead of sample extract. The percentage α-amylase inhibitory activity was calculated with the same equation as for percentage inhibition in the DPPH radical inhibition assay.

4.2.4.6 α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was determined by an assay modified from McCue *et al*. (2005). α-Glucosidase was assayed by using 50 µL of sample extracts and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 U/mL) and was incubated in 96-well plates at 25°C for 10 min. After preincubation, 50 µL of 5 mM p-nitrophenyl-α-d-glucopyranoside solution in 0.1 M phosphate buffer (pH

6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, absorbance readings were recorded at 405 nm by a microplate reader (Thermomax, Molecular Devices Co., Sunnyvale, CA) and compared to a control that had 50 µL of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated with the same equation as for percentage inhibition in the DPPH radical inhibition assay. Dose dependency was tested using 25 µl and 10 µl of the sample, the volume made up to 50 µl using 0.1 M phosphate buffer (pH 6.9) and same protocol was followed.

4.2.4.7 ACE Inhibition Assay

ACE inhibition was assayed by a method modified by Kwon *et al.* (2006). The substrate hippuryl-histidyl-leucine (HHL) and the enzyme ACE-I from rabbit lung (EC 3.4.15.1) were used. Fifty µL of sample extracts were incubated with 100 µL of 1 M NaCl-borate buffer (pH 8.3) containing 2 mU of ACE-I solution at 37°C for 10 min. After preincubation, 100 µL of a 5 mU substrate (HHL) solution was added to the reaction mixture. Test solutions were incubated at 37°C for 1 hour. The reaction was stopped with 150 µL of 0.5 N HCl. Five µL of the sample was injected in a high-performance liquid chromatography (HPLC) apparatus (Agilent 1100 series equipped with autosampler and DAD 1100 diode array detector, Agilent Technologies, Palo Alto, CA). The solvents used for gradient were (1) 10 mM phosphoric acid (pH 2.5) and (2) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for 5 min and then was decreased to 0% for the next 5 min (total run time, 18

min). The analytical column used was an Agilent Nucleosil 100-5C18, 250 mm × 4.6 mm inside diameter, with packing material of 5 μm particle size at a flow rate of 1 mL/min at ambient temperature. During each run, the absorbance was recorded at 228 nm, and the chromatogram was integrated using the Agilent Chemstation (Agilent Technologies) enhanced integrator for detection of liberated hippuric acid (A). Hippuric acid standard was used to calibrate the standard curve and retention time. The percentage inhibition was calculated by:

$$\% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{extract}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100$$

4.2.4.8 HPLC Analysis of Phenolic Phytochemicals

Two milliliters of the extracts was filtered (pore size, 0.2 μm), and 5 μL was injected in the HPLC apparatus (Agilent 1100 series equipped with autosampler and DAD 1100 diode array detector). The solvents used for gradient elution were (A) 10 m*M* phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% over the next 7 minutes, then decreased to 0% for the next 3 minutes, and maintained for the next 7 minutes (total run time, 25 minutes). The analytical column used was an Agilent Zorbax SB-C18, 250 mm × 4.6 mm i.d., with packing material of 5 μm particle size at a flow rate of 1 mL/minute at ambient temperature. During each run the absorbance was recorded at 306 nm and 333 nm, and the chromatogram was integrated using the Agilent Chemstation enhanced integrator. Calibration was performed by injecting the standards of cinnamic acid, rosmarinic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid,

ferulic acid, and quercetin. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards. The results were expressed as μg/g of sample DW.

4.2.4.9 Statistical Analysis

All experiments were performed in either duplicates or triplicates. Analysis at every time point from each experiment was carried out in duplicate or triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP.

4.2.5 Results and Discussion

4.2.5.1 Total Soluble Phenolics and Total Antioxidant Activity by DPPH

In this study, we focused on aqueous and ethanolic extracts from seeds of six different species from the family Apiaceae, to screen their anti-hyperglycemia and anti-hypertension potentials using *in vitro* assays. Figure 10 shows the total phenolic content of aqueous seed extracts. Total phenolic content for these samples ranged from 4.8 mg/g (Coriander) to 8.3 mg/g (Ajowan) of sample DW. Figure 11 shows total phenolic content of ethanolic extracts, which ranged from 2.42 mg/g (Caraway) to 8 mg/g (Ajowan) of sample DW. Plant phenolic content is known to be linked to therapeutic potential for use in prevention and management of type II diabetes. Medicinal plant species observed in this study could be considered a good source of polyphenols due to their high total phenolic content (Kesilova *et al*., 2006), and related total antioxidant activity. The moderate and high correlations in this study suggest that, high antioxidant potential varied for each species.

Figure 10 shows results for antioxidant activity by DPPH assay for aqueous extracts. *In vitro* antioxidant activity measured by DPPH assay for aqueous extracts ranged from 62.8% (Caraway) to 76.5% (Dill). Total phenolic content of aqueous samples indicated a moderate correlation ($r = 0.61$) with antioxidant activity by DPPH assay. Addition of antioxidants to the diet has potential for slowing down oxidation of fats (Yen and Duh, 1994), and can be identified as free radical or active oxygen scavengers (Oktay *el al*., 2003). Therefore, high DPPH inhibition observed for aqueous

extracts suggest that inclusion of these seed ingredients from food condiment sources in our diet could have relevance for combating chronic oxidation-linked metabolic pathways associated with high calorie diet and associated obesity. This could potentially serve as an important factor for controlling obesity which is linked to the incidence of type II diabetes.

Figure 11 shows the results of ethanolic extracts for all samples, which ranged from 25.1% (Coriander) to 68.7% (Ajowan). Total phenolic content of ethanolic extracts had a high correlation ($r = 0.73$) with antioxidant activity by DPPH. This high correlation suggests that the amount of antioxidants in ethanolic extracts is due to the phenolic content of plant species as suggested by Kesilova *et al*. (2006) and many other studies. Specific species with high antioxidant activity from family Apiaceae such as dill and ajowan could be part of food preparation as condiments in foods for managing chronic disease states or even as medicinal material for replacing synthetic antioxidants. Synthetic antioxidants are also being restricted due to their carcinogenicity (Zheng and Wang, 2001), and natural antioxidants have the capability of both preserving food, adding flavor and potentially protecting human body from free radicals and associated chronic diseases (Oktay *et al*., 2003).

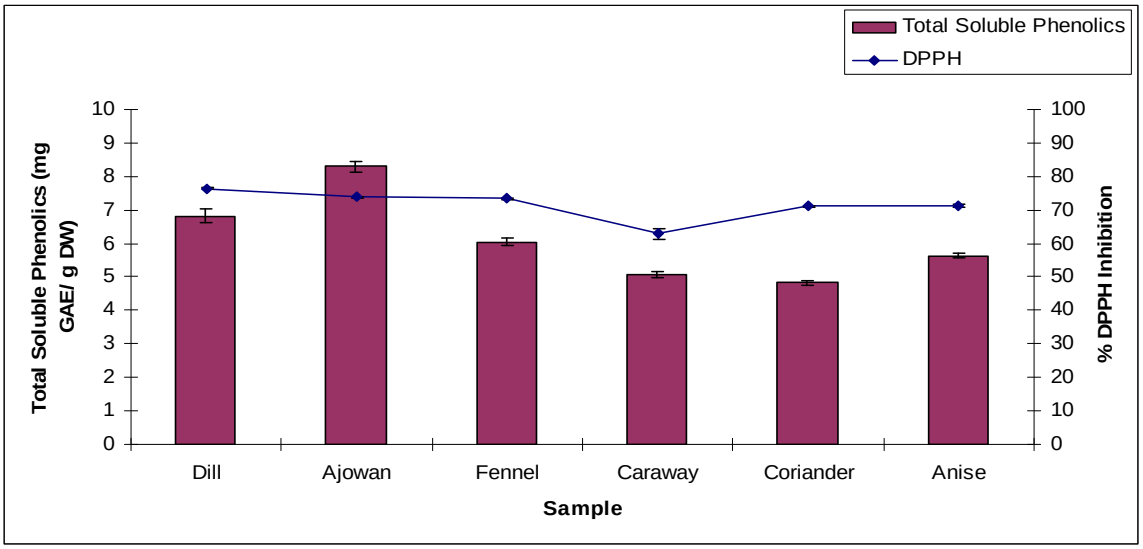

Figure 10 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of aqueous extracts of Dill, Ajowan, Fennel, Caraway, Coriander and Anise.

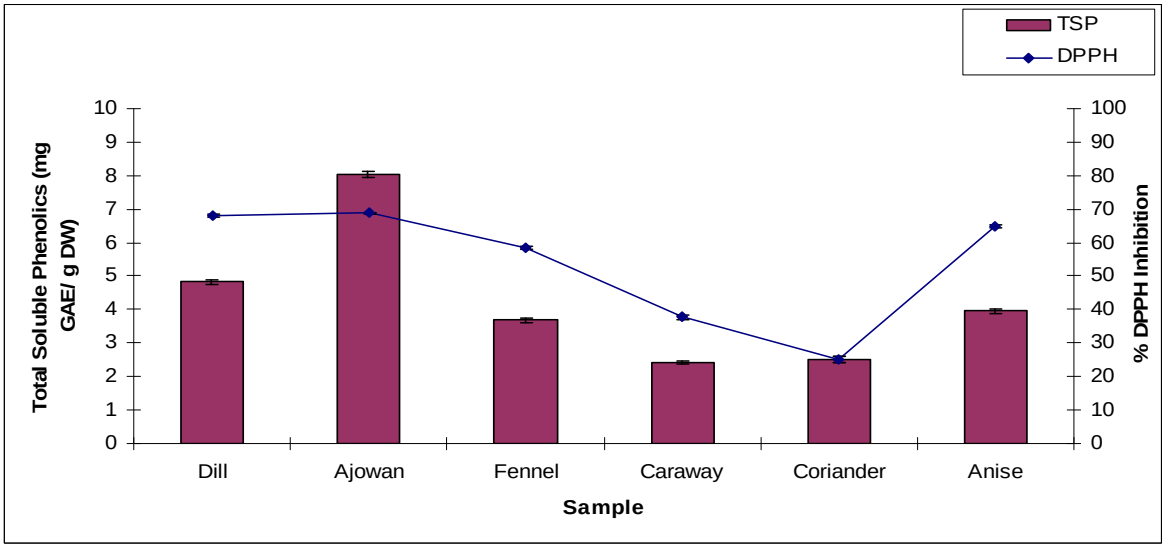

Figure 11 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of ethanolic extracts of Dill, Ajowan, Fennel, Caraway, Coriander and Anise.

4.2.5.2 α-Glucosidase Inhibition

Dose dependency studies (10μL, 25μL, 50μL) for the 6 species of family Apiaceae demonstrated dose dependent responses. Figure 12 illustrates the results for aqueous extracts, which ranged from 0% inhibition (Coriander and Anise) to almost 50% inhibition (Dill). The total phenolic content for aqueous extracts showed a high correlation ($r = 0.86$) with α-glucosidase inhibition. Also, the total phenolic content for ethanolic extracts showed a high correlation ($r = 0.76$) with α-glucosidase inhibition. Natural herbal medicines have been targeted all over the world for prevention of high blood pressure and type II diabetes (Loizzo *et al*., 2008). α-Glucosidase inhibition results from this study suggest that family Apiaceae consists of certain hypoglycemic bioactive compounds. This inhibition of digestive enzyme α-glucosidase would delay the degradation of oligosaccharides, which would decrease the absorption of glucose inhibiting the increase in postprandial hyperglycemia (Loizzo *et al*., 2008). High antioxidant activity and total phenolic content appeared to be a good predictor of α-glucosidase inhibition because of its high correlation for aqueous extracts. Therefore condiments with high α-glucosidase inhibition such as Dill and Ajowan could be used as condiment target species from family Apiaceae to be part of dietary management of hyperglycemia linked to type II diabetes.

Figure 13 shows the results of α-glucosidase inhibition for ethanolic extracts. Unlike aqueous extracts, where samples Coriander and Anise showed no inhibition, for ethanolic extracts there was some inhibition for all 6 samples. α-Glucosidase inhibition for ethanolic extracts ranged from 1.7% (Anise) to 32% (Ajowan). Antioxidant activity

for aqueous extracts showed a low correlation ($r = 0.48$) with α-glucosidase inhibition. In terms of antioxidant activity ethanolic extracts showed a moderate correlation ($r = 0.55$) with α-glucosidase inhibition. Overall, α-glucosidase inhibition observed for ethanolic extracts is lower than aqueous extracts, but suggests that they still contain hypoglycemic bioactive compounds required for potentially managing hyperglycemia. We suspect that higher inhibition of aqueous extracts could be a more natural way of preparing food, and preparation with hot water might be more efficient for the outcome to naturally reach digestive enzymes to manage hyperglycemia. Based on these results, seed sources of plant species such as family Apiaceae has the potential for anti-diabetic activity which could prove to be effective as the clinical agents when consumed in small doses on a consistent basis through the diet. Overall high antioxidant activity and total phenolic content did not prove to be a good indicator of α-glucosidase inhibition because of low to moderate correlations for both aqueous and ethanol extracts.

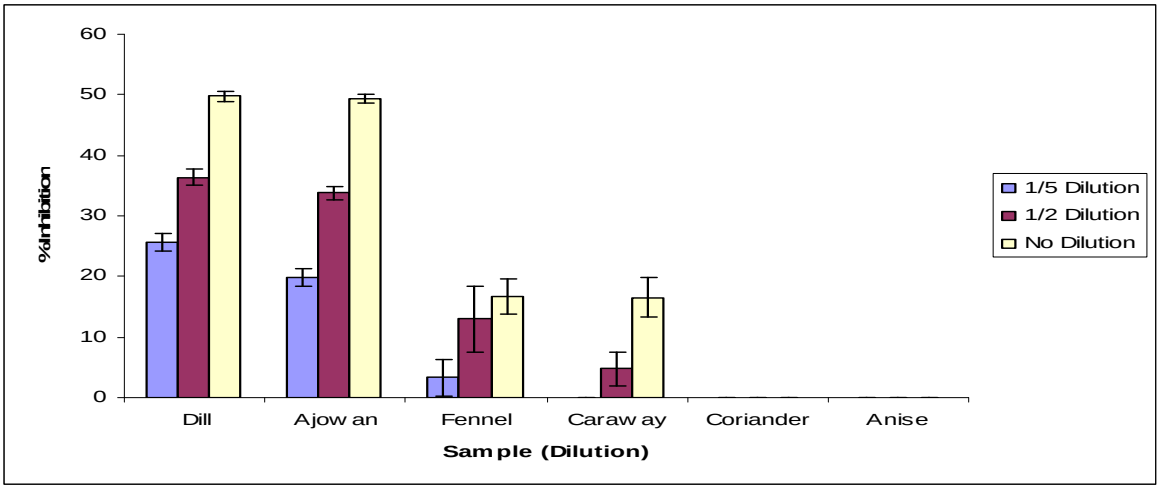

Figure 12 Changes observed in dose dependent (10 µL, 25 µL, 50 µL) % α-glucosidase inhibitory activities for aqueous extracts of Dill, Ajowan, Fennel, Caraway, Coriander and Anise.

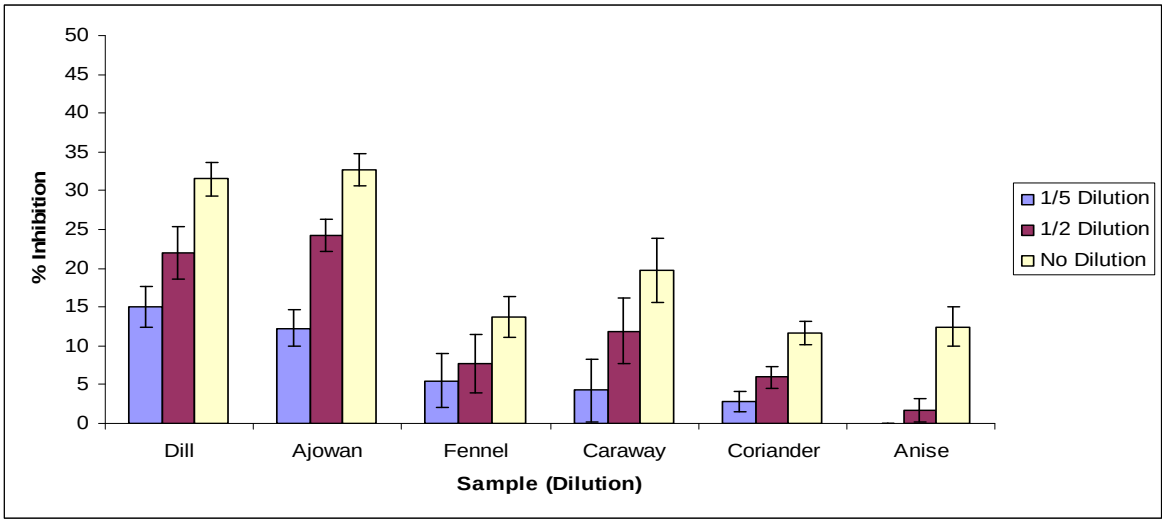

Figure 13 Changes observed in dose dependent (10 µL, 25 µL, 50 µL) % α-glucosidase inhibitory activities for ethanolic extracts of Dill, Ajowan, Fennel, Caraway, Coriander and Anise.

4.2.5.3 α- Amylase Inhibition

Dose dependency studies (10µl, 25µ1, 50µl) allowed us to observe dose dependent responses for the 6 species of family Apiaceae. Figure 14 shows results for aqueous extracts, which were in the range from 0% (Ajowan) to 32% (Caraway). Total phenolic content for aqueous extracts demonstrated an inverse correlation ($r = -0.54$) with α-amylase inhibition. Also, phenolic content for ethanolic extracts showed an inverse correlation ($r = -0.66$) with α- amylase inhibition. Above correlations observed, indicated that total phenolic content has no influence on α-amylase inhibition. Previous studies have suggested that α-amylase inhibition is not linked to high antioxidant activity or total phenolics content (Cheplick *et al*., 2010).

Aqueous extracts for samples of this study indicate a slightly higher inhibition than ethanolic extracts. Even though α-amylase inhibition is considered to have positive affects on prevention of hyperglycemia linked to type II diabetes, excess α- amylase inhibition could lead to stomach distention and discomfort (Puls *et al*., 1977; Cheplick *et al*., 2010). Moderate α-amylase inhibition and good α-glucosidase inhibition makes family Apiaceae species good candidates for managing early stage hyperglycemia linked to type II diabetes.

Figure 15 shows results for ethanolic extracts, which ranged from 0% (Ajowan) to 30% (Coriander). Antioxidant activity for aqueous extracts showed an inverse correlation ($r = -0.54$) with α-amylase inhibition. Also, antioxidant activity for ethanolic extracts demonstrated an inverse correlation ($r = -0.66$) with α-amylase inhibition. Correlations between α-amylase and DPPH inhibition suggest that, total antioxidant activity does not reflect the potential for α-amylase inhibition. This indicates that overall phenolic antioxidants do not play any role in determining α-amylase inhibitory potential. It is also possible that specific phenolics not present in species of family Apiaceae studied maybe responsible or as suggested in previous studies (Kwon et al., 2006; Cheplick et al., 2010) non-phenolic bioactives may be responsible. In general, managing hyperglycemia linked to type II diabetes can be targeted through moderate α-amylase inhibition along with α-glucosidase inhibition (Pinto *et al*., 2008). Therefore, Apiaceae family species such as Dill and Ajowan might be considered good candidates for further *in vivo* studies to gain a better insight on managing early stage hyperglycemia linked to type II diabetes. α-Amylase inhibition of family Apiaceae, such as Dill and Caraway in aqueous and ethanolic extracts also have potential of controlling blood glucose level in the context of

preventing and managing type II diabetes through combinations of these condiments with other whole foods with similar bioactive properties as in the case of Chilean potatoes.

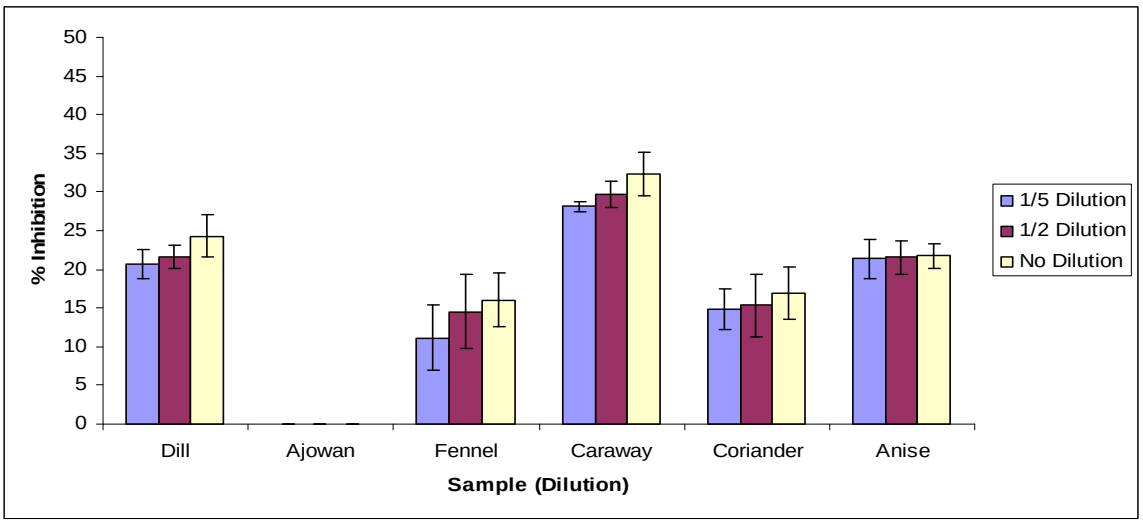

Figure 14 Changes observed in dose dependent (10 µL, 25 µL, 50 µL)) % α-amylase inhibitory activities for aqueous extracts of Dill, Ajowan, Fennel, Caraway, Coriander and Anise.

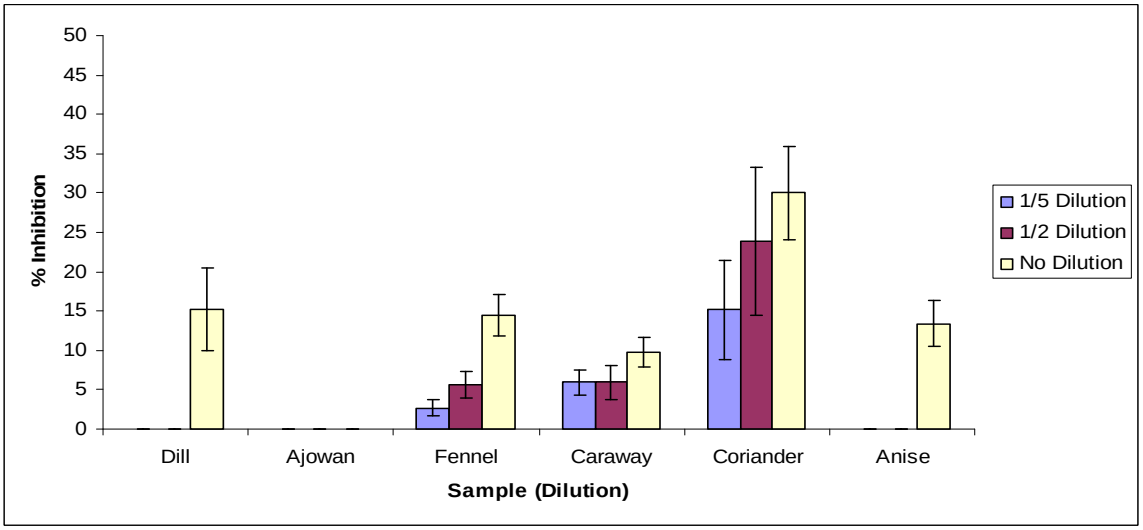

Figure 15 Changes observed in dose dependent (10 µL, 25 µL, 50 µL)) % α-amylase inhibitory activities for ethanolic extracts of Dill, Ajowan, Fennel, Caraway, Coriander and Anise.

4.2.5.4 ACE Inhibition

Hypertension is known to be a risk factor of various cardiovascular diseases and is associated with long term diabetes (Kwon *et al*., 2006). In this study, we did not observe ACE inhibitory activity for neither aqueous nor ethanolic extracts for family Apiaceae. We suspect that, reason behind lack of ACE inhibition could be the use of smaller amount of sample (2.5 g) to prepare extracts.

4.2.5.5 HPLC Analysis of Phenolic Phytochemicals

We observed 8 phenolic compounds in 6 selected species of family Apiaceae based on the phenolic profile analysis using HPLC. The 8 phenolic compounds observed include caffeic acid, catechin, rutin, chlorogenic acid, gallic acid, ferulic acid and rosmarinic acid. The amounts (mg/g DW) and their standard error values are presented in Table 4 and 5 for water and ethanol extracts. The content of caffeic acid in aqueous extracts ranged from 0 mg/g (Coriander and Anise) to 3.93 mg/g (Fennel) of sample DW. Caffeic acid in ethanolic extracts ranged from 0 mg/g (Dill and Ajowan) to 5.53 mg/g (Anise) of sample DW. These results suggest that ethanolic and aqueous extracts have specific phenolics in different species and in specific cases higher in ethanolic extracts. Anise seeds do not have any bioactive caffeic acid in aqueous extract (0 mg/g DW) but has higher content in its ethanolic extract (5.53 mg/g DW). Caffeic acid is known to be naturally present in fruits and vegetables, and has anti-inflammatory and anti-oxidative properties (Son and Lewis, 2002). Therefore, we can relate the actions of caffeic acid to

potentially help manage chronic oxidative diseases such as type II diabetes and cardiovascular diseases.

The catechin content in aqueous extracts of various samples ranged from 0 mg/g (Fennel, Caraway, Coriander and Anise) to 2.23 mg/g (Ajowan) of sample DW. Catechin activity for ethanolic extracts ranged from 0 mg/g (Fennel, Caraway and Anise) to 1.26 mg/g (Ajowan) of sample DW. Affect of catechin on human health has attracted attention due to its antioxidant potential. It has been shown that ingestion of catechins is known to decrease waist size while reducing body fat (Nagao *et al*., 2007). Even though the amount of catechin observed is low, specific species from family Apiaceae has potential of affecting energy and fat metabolism when incorporated in diet designs on consistent basis along with other legumes, fruits and vegetables containing catechins.

The content of rutin in aqueous extracts ranged from 0 mg/g (Caraway and Anise) to 27.6 mg/g (Dill) of sample DW. Rutin content in ethanolic extracts ranged from 0 mg/g (Fennel, Caraway and Anise) to 17.8 mg/g (Dill) of sample DW. The content of Dill is the highest of any phenolic compounds found in the current study. Rutin is known to have affects on dilating blood vessels and improving interpenetration of veins (Wang *et al*., 2003). Rutin is found in many plants and is reported to have anti-inflammatory and antioxidant activities. Rutin's antioxidant activity is known to be responsible for many of its bioactive activities (La Casa *et al*., 2000), such as preventing oxidative stress in pancreatic beta cells which could lead to uncontrolled proliferation of damaged pancreatic beta cells, increasing diabetes risk (Heineke *et al*., 1993). High amount of rutin present in Dill species leads to the conclusion that, these samples could prove to have protective affects against the development of type II diabetes and further *in vivo* studies

would be needed based on incorporation of such condiments in a range of whole foods, including potato and starch foods such as bread.

The content of chlorogenic acid in aqueous extracts ranged from 0 mg/g (Dill, Ajowan and Anise) to 4.71 mg/g (Coriander) of sample DW. Chlorogenic acid content in ethanolic extracts was only observed for anise, which was 2.78 mg/g of sample DW. Chlorogenic acid is an antioxidant and potentially contributes in the prevention of cardiovascular diseases and type II diabetes. The content of gallic acid was observed in Caraway and Anise for aqueous extracts, which were 0.13 mg/g and 0.09 mg/g of sample DW, respectively. The content of gallic acid in Caraway and Coriander for ethanolic extracts was 1.02 mg/g and 0.55 mg/g of sample DW, respectively. Higher content of gallic acid was found in ethanolic extracts of Caraway and Coriander than aqueous extracts, but in general, a higher overall concentration was observed in aqueous extracts of other species in this study. The content of p-coumaric acid and ferulic acid was very low as indicated in Table 5. The content of rosmarinic acid in aqueous extracts ranged from 0 mg/g (Fennel and Coriander) to 7.08 mg/g (Ajowan) of sample DW. The content of rosmarinic acid in ethanolic extracts ranged from 0 mg/g (Caraway and Coriander) to 7.18 mg/g (Dill) of sample DW. Rosmarinic acid is a well known bioactive phenolic compound targeted against oxidation-linked diseases (Shetty, 1997; Shetty and Wahlqvist, 2004).

Presence of all the above phenolic compounds in seeds suggests that select species of family Apiaceae in aqueous and ethanolic extracts have ability to provide protection against oxidation-linked diseases. Such seed ingredients in whole food form

can be used as condiments with a range of food designs for better dietary management of hyperglycemia linked to type II diabetes.

Table 4 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Family Apiaceae for Aqueous and Ethanolic Extracts. Analysis of Phenolic Compounds: Caffeic Acid, Catechin, Rutin and Chlorogenic Acid.

Sample	Caffeic Acid	Catechin	Rutin	Chlorogenic Acid
	mg/g DW		mg/g DW	mg/g DW
H2O				
Dill	1.62 ± 0.10	2.22 ± 0.14	27.6 ± 1.84	_____
Ajowan	1.14 ± 0.03	2.23 ± 0.03	4.00 ± 0.52	_____
Fennel	3.93 ± 0.03	_____	5.37 ± 0.04	4.69 ± 0.03
Caraway	0.69 ± 0.02	_____	_____	2.99 ± 0.01
Coriander	_____	_____	5.50 ± 0.15	4.71 ± 0.06
Anise	_____	_____	_____	_____
ETHANOL				
Dill	_____	1.12 ± 0.40	17.8 ± 6.20	_____
Ajowan	_____	1.26 ± 0.23	3.26 ± 0.86	_____
Fennel	1.56 ± 0.121	_____	_____	_____
Caraway	0.88 ± 0.00	_____	_____	_____
Coriander	0.90 ± 0.33	0.13 ± 0.00	1.25 ± 0.11	_____
Anise	5.53 ± 2.07	_____	_____	2.78 ± 0.00

Table 5 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Family Apiaceae for Aqueous and Ethanolic Extracts. Analysis of Phenolic Compounds: Gallic Acid, P-coumaric Acid, Ferulic Acid and Rosmarinic Acid.

Sample	Gallic Acid	p-coumaric acid	Ferulic Acid	Rosmarinic Acid
	mg/g DW	mg/g DW	mg/g DW	mg/g DW
H2O				
Dill	_____	_____	_____	2.96 ± 0.18
Ajowan	_____	_____	_____	7.08 ± 3.92
Fennel	_____	_____	_____	_____
Caraway	0.13 ± 0.00	_____	_____	1.09 ± 0.31
Coriander	_____	0.15 ± 0.01	1.98 ± 0.03	_____
Anise	0.09 ± 0.01	_____	_____	2.84 ± 0.00
ETHANOL				
Dill	_____	_____	_____	7.18 ± 0.00
Ajowan	_____	_____	_____	2.32 ± 0.49
Fennel	_____	_____	_____	1.65 ± 0.00
Caraway	1.02 ± 0.48	0.11 ± 0.00	_____	_____
Coriander	0.55 ± 0.03	0.24 ± 0.00	0.69 ± 0.00	_____
Anise	_____	0.82 ± 0.00	_____	5.54 ± 1.74

4.2.6 Conclusions

In this study, we have evaluated phenolic-linked anti-hyperglycemia potential of seed phytochemicals of 6 select species of family Apiaceae with relevance for dietary management of type II diabetes. Hot water and 12% ethanol extraction methods were utilized to prepare samples used for further evaluation of their functionalities, using *in vitro* assays. Total Soluble Phenolics, DPPH inhibition assay, α-glucosidase inhibition, α-amylase inhibition and ACE inhibition were evaluated. Results indicated that there was no ACE inhibition in any of the 6 selected species of family Apiaceae. α-Glucosidase inhibition ranged upto 50%, and showed a large variation among samples. This observation indicates that including Apiaceae family species in our diet would have potential to manage hyperglycemia contributing potentially to overall prevention and management of type II diabetes. Further, α-amylase inhibition was observed upto 30%, in some species which indicates that select species from Apiaceae family could be helpful towards controlling blood glucose levels preventing hyperglycemia. This research study provides *in vitro* biochemical rationale of disease-linked functions of select seed phytochemicals of the family Apiaceae, which provides the structure-function basis for *in vivo* experiments for development of therapeutic strategies to help prevent chronic hyperglycemia and associated complications linked to management of type II diabetes.

4.3 Anti-Diabetic Potential of Select Middle Eastern Herbs of Family Lamiaceae Using *In Vitro* Assays

4.3.1 Abstract

Emerging global trends indicate the use of bioactive compounds from plants as sources of food and medicine for prevention and management of type II diabetes. In this study, we screened 2 plant species of Lamiaceae family from the Near East Asia for its total phenolic content and total antioxidant activity. *In vitro* functionality assays were used to screen for α-glucosidase, α-amylase and angiotensin converting enzyme (ACE) inhibitory activities to explore the biochemical relevance for management of hyperglycemia and hypertension. Aqueous extracts of marjoram (41.1 mg/g DW) and sage (38.4 mg/g DW) had higher total phenolic content compared to ethanolic extracts of 22.1 mg/g (Marjoram) and 16.7 mg/g (Sage). High α-glucosidase inhibitory activity was observed for aqueous extracts of marjoram (86%) and sage (77%). High α-glucosidase inhibition suggests that species from Lamiaceae family has potential to manage hyperglycemia linked to type II diabetes.

4.3.2 Introduction

Type II diabetes is considered as one of the most common metabolic disorders worldwide and management of this epidemic is now targeting wide range of plants used in food and medicine from local bioresources. Today, as many as 80% of the world population relies on using traditional medicine for healthcare purposes (Muthu *et al*., 2006). In the Middle East, wild edible plants have been used as a source of food and medicine since ancient times. People from the Middle Eastern origin believe there is a strong relationship between food and medicine (Dogan *et al*., 2004). Natural α-amylase and α-glucosidase inhibitors from food grade plants offer cost effective and locally based strategies to control postprandial hyperglycemia, and these inhibitors could be used in therapies with minimum side effects (Kwon *et al*., 2006; Pinto et al., 2009). Plant sources used as medicinal herbs have good potential over synthetic drugs due to low toxicity of plants (Kwon *et al*., 2006).

Herbs are a rich source of phenolic phytochemicals having high antioxidant activity (Kwon *et al*., 2006). High phenolic antioxidants from plants are highly correlated with natural α-glucosidase inhibitors, and its presence increases the potential for preventing hyperglycemia linked to on-set of type II diabetes. Plants such as *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage) belongs to Lamiaceae family (Mint Family), which also includes other plants such as rosemary and lavenders. Plants from Lamiaceae family have commonly been used for food preservation, culinary flavors and for treating common illnesses as traditional medicine (Shetty, 1997; Kwon *et al*., 2006). Many plants and herbs related to Lamiaceae family have originated from the Middle East

region in Asia. Lamiaceae family has aromatic characteristics and most of them are grown for their essential oil producing tendencies. These herbs contain phenolic phytochemicals, having therapeutic properties that are beneficial towards human health (Shetty, 1997). Phenolic phytochemicals are associated with having potential for managing chronic oxidation linked diseases, such as cardiovascular diseases and diabetes (Shetty, 1997; Pinto and Shetty, 2010). Also, phytochemicals contain significant antioxidant capacities which have potential to lower mortality rates of cancer in human populations (Veioglu *et al*., 1998).

Free radicals play an important role in developing tissue damage in several diseases such as metabolic syndrome diseases, cancer, neurodegenerative diseases and pathological disorders (Erdemoglu *et al*., 2006). Antioxidants are known to play a crucial part in prevention of many such diseases. Antioxidants inhibit the oxidation of lipids, by inhibiting the propagation of oxidative chain reactions (Javanmardi *et al*., 2003). Medicinal plants and herbs such as those species in the family Lamiaceae are an excellent source of natural antioxidants used as spices and aromatic herbs. In particular, plants such as sage, marjoram, thyme and rosemary are known to have strong antioxidant activity. Salvia is the genera of plants that is considered one of the most diverse in Turkey with many species, and has been used as traditional medicine to treat common colds and stomach disorders (Cuvelier *et al*., 1996). Water extracts of sage are commonly used in the Middle East to treat common colds, coughs and as anti-inflammatory agent in oral cavity (Farhat *et al*., 2001). Studies have shown that essential oils of this plant has antibacterial effects, as well as suppressive activities against tumor formation (Hilan *et al*., 1997; Farhat *et al*., 2001) *Origanum majorana* commonly known as marjoram, is also

rich in essential oils and also characterized with high content of phenolic compounds. Marjoram is commonly used in the Middle East as folk medicine, particularly in the form of tea as a prescription for fever, sinus congestion and nervous disorders (Qari, 2008). Marjoram is known for its potent antioxidant and antimicrobial activities similar to sage. Marjoram consists of anti-hepatoma and anti-genotoxicity activities, based on the indication that it reduces number of cell and chromosomal aberrations (Qari, 2008). These characteristics of marjoram could play an important preventive role in occurrence of type II diabetes. Pancreatic beta cells could be damaged due to oxidative stress before they are replicated or proliferated. If damaged pancreatic beta cells are prevented from proliferating through cell repair and apoptosis, then the possibility of incidence of diabetes from occurring could be reduced. Therefore, the inclusion of herbal food sources in our diet could help manage hyperglycemia in early stages which is linked to long term diabetes from further defects in pancreatic cells from hyperglycemia-linked oxidative damage.

Therefore the objective of this research study was to screen 2 species belonging to Lamiaceae family from the Near East Asia region for their total phenolic content and total antioxidant activity. Following these studies, *in vitro* assays such as α-amylase, α-glucosidase and ACE inhibitory activities were evaluated. These *in vitro* assays would provide biochemical rationale for use of sage and marjoram in prevention of hyperglycemia and hypertension linked to type II diabetes. Also, we determined individual phenolic compounds found in phenolic profile by HPLC. This would allow us to correlate phenolic compounds of herbs to total antioxidant activity and total soluble phenolics content and link it to specific health relevant functional activity.

4.3.3 Materials and Methods

4.3.3.1 Materials

Dried herb sample of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage) were obtained from the Department of Biology of United Arab Emirates University, Al-Ain, UAE. Porcine pancreatic α-amylase (EC 3.2.1.1), rat intestinal α-glucosidase (EC 3.2.1.20), hippuric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), rabbit lung ACE (EC 3.4.15.1), cinnamic acid, rosmarinic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO).

4.3.3.2 Sample Preparation

Water Extracts:

The samples (2.5 g) were extracted in 100 mL of distilled water under reflux at 95°C for 30 minutes. The samples were centrifuged for 10 minutes and stored in a refrigerator at -20°C until analysis, for no more than 1 week.

Ethanol extracts:

The samples (2.5 g) were extracted in 100 mL of 12% ethanol in a shaker at a speed of 150 RPM overnight at 20°C. The samples were filtered and stored in a refrigerator at –20°C until analysis, for no more than 1 week.

4.3.3.3 Total Phenolic Assay

The total phenolics in all samples were determined by using a method modified by Shetty *et al.* (1995). In brief, 0.5 mL of sample extract was added to a test tube and mixed with 0.5 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (vol/vol) Folin-Ciocalteu reagent was added and mixed. The absorbance was read at 725 nm using a spectrophotometer (Genesys UV/Visible, Milton Roy, Inc., Rochester, NY). Different concentrations of gallic acid were used to develop a standard curve. Results were expressed as mg of gallic acid/g of sample dry weight (DW).

4.3.3.4 Antioxidant Activity by DPPH Radical Inhibition Assay

The antioxidant activity was determined by the DPPH radical scavenging method modified from Kwon *et al.* (2006). A 250-μL aliquot of the sample extract was mixed with 1,250 μL of DPPH (60 μM in ethanol). The mixture was centrifuged at 13,000 *g* for 1 minute, and after this the absorbance was measured at 517 nm using the Genesys UV/Visible spectrophotometer. The readings were compared with the controls, containing 95% ethanol instead of sample extract. The percentage inhibition was calculated by:

$$\%\ \text{inhibition} = \frac{(Absorbance_{\text{control}} - Absorbance_{\text{extract}})}{Absorbance_{\text{control}}} \times 100$$

4.3.3.5 α-Amylase Inhibition Assay

The α-amylase inhibitory activity was determined by an assay modified from the *Worthington Enzyme Manual* (Worthington, 1993). A total of 500 µL of sample extract and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25°C for 10 minutes. After preincubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted after adding 5-15 mL of distilled water, and the absorbance was measured at 540 nm using the Genesys UV/Visible spectrophotometer. The readings were compared with the controls, containing buffer instead of sample extract. The percentage α-amylase inhibitory activity was calculated with the same equation as for percentage inhibition in the DPPH radical inhibition assay.

4.3.3.6 α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was determined by an assay modified from McCue *et al.* (2005). α-Glucosidase was assayed by using 50 µL of sample extracts and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 U/mL) and was incubated in 96-well plates at 25°C for 10 min. After preincubation, 50 µL of 5 mM p-nitrophenyl-α-d-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was

added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, absorbance readings were recorded at 405 nm by a microplate reader (Thermomax, Molecular Devices Co., Sunnyvale, CA) and compared to a control that had 50 μL of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated with the same equation as for percentage inhibition in the DPPH radical inhibition assay. Dose dependency was tested using 10 μL and 25 μL of the sample, the volume made up to 50 μL using 0.1 M phosphate buffer (pH 6.9) and same protocol was followed.

4.3.3.7 ACE Inhibition Assay

ACE inhibition was assayed by a method modified by Kwon *et al.* (2006). The substrate hippuryl-histidyl-leucine (HHL) and the enzyme ACE-I from rabbit lung (EC 3.4.15.1) were used. Fifty μL of sample extracts were incubated with 100 μL of 1 M NaCl-borate buffer (pH 8.3) containing 2 mU of ACE-I solution at 37°C for 10 min. After preincubation, 100 μL of a 5 mU substrate (HHL) solution was added to the reaction mixture. Test solutions were incubated at 37°C for 1 hour. The reaction was stopped with 150 μL of 0.5 N HCl. Five μl of the sample was injected in a high-performance liquid chromatography (HPLC) apparatus (Agilent 1100 series equipped with autosampler and DAD 1100 diode array detector, Agilent Technologies, Palo Alto, CA). The solvents used for gradient were (1) 10 mM phosphoric acid (pH 2.5) and (2) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for 5 min and then was decreased to 0% for the next 5 min (total run time, 18

min). The analytical column used was an Agilent Nucleosil 100-5C18, 250 mm × 4.6 mm inside diameter, with packing material of 5 μm particle size at a flow rate of 1 ml/min at ambient temperature. During each run, the absorbance was recorded at 228 nm, and the chromatogram was integrated using the Agilent Chemstation (Agilent Technologies) enhanced integrator for detection of liberated hippuric acid (A). Hippuric acid standard was used to calibrate the standard curve and retention time. The percentage inhibition was calculated by:

$$\% \text{ inhibition } = \frac{(A_{\text{control}} - A_{\text{extract}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100$$

4.3.3.8 HPLC Analysis of Phenolic Phytochemicals

Two milliliters of the extracts was filtered (pore size, 0.2 μm), and 5 μL was injected in the HPLC apparatus (Agilent 1100 series equipped with autosampler and DAD 1100 diode array detector). The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% over the next 7 minutes, then decreased to 0% for the next 3 minutes, and maintained for the next 7 minutes (total run time, 25 minutes). The analytical column used was an Agilent Zorbax SB-C18, 250 mm × 4.6 mm i.d., with packing material of 5 μm particle size at a flow rate of 1 mL/minute at ambient temperature. During each run the absorbance was recorded at 306 nm and 333 nm, and the chromatogram was integrated using the Agilent Chemstation enhanced integrator. Calibration was performed by injecting the standards of cinnamic acid, rosmarinic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid,

ferulic acid, and quercetin. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards. The results were expressed as μg/g of sample DW.

4.3.3.9 Statistical Analysis

All experiments were performed in either duplicates or triplicates. Analysis at every time point from each experiment was carried out in duplicate or triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP.

4.3.4 Results and Discussion

4.3.4.1 Total Phenolics and Antioxidant Activity by DPPH Inhibition

In this study, we focused on aqueous and ethanolic extracts of two species common to Near East regions of Asia belonging to Lamiaceae family. Samples were screened for their anti-hyperglycemia and anti-hypertension potential based on *in vitro* assays relevant to development of biochemical rationale for management of type II diabetes in the early stages. Figure 16 shows the total phenolic content of aqueous extracts for *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage). Total phenolic content for aqueous extract of marjoram was observed to be 41.15 mg/g of sample dry weight (DW), and sage was 38.4 mg/g of sample DW. Total phenolic content observed for ethanolic extracts of sage and marjoram was lower than that observed for aqueous extracts. High concentration of phenolic phytochemicals observed in oregano previously suggests that it has high antioxidant and antimicrobial activity (Chun *et al*., 2005). Figure 17 shows phenolic content for ethanolic extracts of marjoram (22.15 mg/g of sample DW) and sage (16.7 mg/g of sample DW). Extraction process for aqueous extracts was carried out under higher temperatures than ethanolic extracts, and therefore resulted in higher extractable phenolic content for marjoram and sage (Seaberg *et al*., 2003; Chun *et al*., 2005). The high phenolic content indicates the relevance for hyperglycemia linked to type II diabetes (Kwon *et al*., 2006). High phenolic content indicates the potential of ingestion of sage and marjoram in various food designs and combinations to slow down and delay in development of type II diabetes.

Total antioxidant activity measured by DPPH inhibition assay indicated similar results for aqueous and ethanolic samples of sage and marjoram, although the phenolic content showed a difference (Figure 16 & 17). The % inhibition of DPPH ranged from 76.6% to 79.6% for aqueous and ethanolic extracts of the samples. This suggests that total phenolic content may not be totally important in determining antioxidant activity of the extracts, but the physico-chemical nature of individual phenolics may play a part (Chun *et al*., 2005). Figure 16 shows the results obtained for total antioxidant activity by DPPH assay for aqueous extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage), with a correlation of total phenolic content. Total antioxidant activity measured for aqueous extracts of marjoram was observed to be 77.5% DPPH inhibition, which was lower than that observed for sage (79%). There was no significant difference seen between the two Lamiaceae samples because of their similar phytochemical compositions, but the polyphenol content was higher in aqueous extracts of sage. Figure 17 shows the total antioxidant activity observed for ethanolic extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage). The % DPPH inhibition measured for ethanolic extracts of marjoram (79.6%) was higher compared to aqueous extracts. Total antioxidant activity observed for sage (76.6%) was lower than the value observed for aqueous extracts. High % DPPH inhibition of sage and marjoram indicates that, total phenolic content in this case cannot be the basis of prediction for total antioxidant activity (Kahkonen *et al*., 1999; Parejo *et al*., 2002). Total phenolic content for aqueous extracts indicated an inverse ($r = -1$) correlation with % DPPH inhibition. But, a high correlation ($r = 1$) was observed between total phenolic content and % DPPH inhibition for ethanolic extracts. High antioxidant activity of sage and marjoram suggests that, plant derived

additives from herbs and spices could prove to be beneficial towards prevention of oxidative stress, and could be potentially beneficial in managing the micro vascular complications of type II diabetes (Kwon *et al*., 2006). Such findings could prove that, substitution of plant extracts for synthetic food antioxidants has potential to influence human health (Martinez-Tome *et al*., 2001; Hinneburg *et al*., 2006). Total phenolic content and total antioxidant activity by DPPH assay indicates that these plants, which are commonly consumed in Mediterranean diet (Parejo *et al*., 2002), can prove to be protective against oxidative stress with cross-over beneficial affects on human health. These herbs could prove to have potential to be industrially efficient due to their high phenolic antioxidant activity, which helps in slowing down oxidative degradation of lipids (Wojdylo *et al*., 2007), and improving the quality and nutritional value of food. High total antioxidant activity measured via DPPH assay also indicates the potential that these antioxidants might play an important part in suppressing oxidative stress caused to pancreatic beta cell function, which may reduce the incidence of type II diabetes (Song *et al*., 2005; Bhandari *et al*., 2008). Therefore, it can be concluded that, specific phenolic compounds responsible for high antioxidant activity may serve as a preventive measure against the development of type II diabetes, if applied through better food designs and combinations for dietary management in early stages.

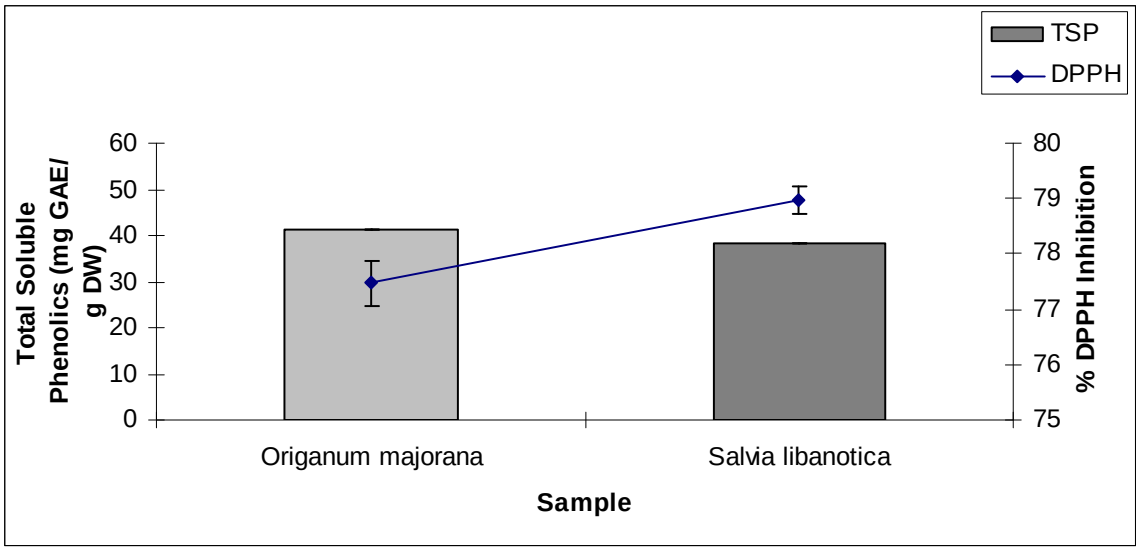

Figure 16 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of aqueous extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage).

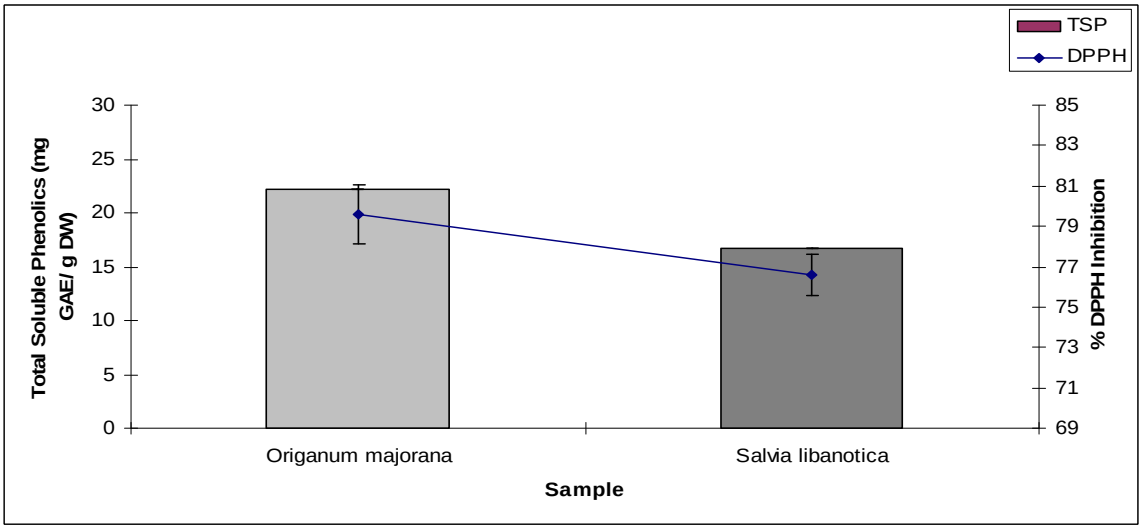

Figure 17 Total Soluble Phenolics (mg GAE/ g DW ± Standard Error) and Total Antioxidant Activity (% DPPH Inhibition ± Standard Error) correlation of ethanolic extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage).

4.3.4.2 α–Glucosidase Inhibition

Type II diabetes is growing rapidly globally mainly due to the diet and lifestyle changes leading to obesity in general population. A therapeutic approach to decrease postprandial hyperglycemia is by slowing the absorption of glucose, by inhibition of α-glucosidase enzyme in digestive system (Bhandari *et al*., 2008). Therefore, we investigated inhibitory activity of α-glucosidase inhibitors in aqueous and ethanolic extracts of sage and marjoram.

Dose dependency studies (10µl, 25µl, 50µl) indicated dose dependent responses, where the highest inhibition was observed for samples with no dilution and the lowest inhibition was seen for 1/5 diluted samples. Therefore as the sample dilution increased, % inhibition was seen to decrease. Figure 18 indicates results for dose dependent studies using extracts of sage and marjoram, which ranged from 55% (Sage, 1/5 dilution sample) to 85.6% (Marjoram, no dilution sample). Aqueous extracts of marjoram indicated higher α-glucosidase inhibitory activity than sage in all dose dependent studies of sample extracts. Aqueous extracts of sage and marjoram had a high α-glucosidase inhibitory activity, which also had a high content of rosmarinic acid (Table 6). Total phenolic content for aqueous extracts showed a high correlation ($r = 1$) with α-glucosidase inhibition. This suggests that there may be some specific phenolic compounds which could be responsible for high α-glucosidase inhibitory activity of the sample extracts. Total phenolic content for ethanolic extracts showed an inverse correlation (r = -1) with α-glucosidase inhibitory activity. Unlike aqueous extracts of the samples, where a high correlation was seen

for total phenolic content and α-glucosidase inhibition, ethanolic extracts showed opposite results with no correlation. The phenolic profile of ethanolic extracts (Table 1), low content of rosmarinic acid was observed, but the content of rutin was observed to be higher for sage compared to aqueous extracts, where no rutin content was found. Therefore, based on the results we can suggest that, Lamiaceae samples with phenolic compound such as rosmarinic acid, caffeic acid and rutin could potentially contain hypoglycemic affects due to their high α-glucosidase inhibitory activities.

Figure 19 shows the results for α-glucosidase inhibition for ethanolic extracts of sage and marjoram. Compared to aqueous extracts of the samples, ethanolic extracts showed higher α-glucosidase inhibitory activity for sage, which ranged from 69% (1/5 sample dilution) to 79% (no sample dilution). These results suggest that, inhibitory activity for sage could potentially be higher in ethanolic extracts due to a certain phenolic compounds being efficiently extracted that are not present in aqueous extracts, such as rutin (Table 6). Inhibitory activity for marjoram was observed to be lower than aqueous extracts, which ranged from 62.6% (1/5 sample dilution) to 70%. Correlations between DPPH assay and α-glucosidase inhibition illustrates an inverse activity ($r = -1$) for both aqueous and ethanolic extracts. This suggests that, antioxidant activity may not totally affect the α-glucosidase inhibitory activity of these Lamiaceae samples, even though the percent inhibitory activities were seen to be higher for both assays. High α-glucosidase inhibitory activity suggests that, sage and marjoram have anti-diabetic potentials observed in both aqueous and ethanolic extracts. Results for sage and marjoram varied for ethanolic and aqueous extracts,

with higher inhibition for marjoram in aqueous extracts and being high for sage in ethanolic extracts. Therefore, we can assume that, enrichment of Lamiaceae herbs in our diet through condiments or other ways could result in enhancement of our health through functional bioactive enrichment of foods. This could be seen in terms of α-glucosidase inhibition relevant to hyperglycemia which is linked to type II diabetes management. Lamiaceae species such as sage and marjoram have high α-glucosidase inhibitory activities for both ethanolic and aqueous extracts, suggesting that the inclusion of these high phenolic herbs in our diet could help reduce blood glucose levels and lengthen the duration of carbohydrate absorption (Ye *et al*., 2002; Kwon *et al*., 2006). Lowering of blood glucose level to normal is the most important part of treating persistent hyperglycemia, which is the characteristic of type II diabetes (Ye *et al*., 2002). Synthetic forms of α-glucosidase inhibitors are known to come with various side effects, and toxicity being one of them, is allowing people to explore alternative strategies, such as developing natural plant food-based strategies through effective dietary management. Therefore, natural forms of α-glucosidase inhibitors present in sage and marjoram could serve potentially to be an important way to suppress elevated blood glucose levels, due to hyperglycemia linked to the incidence of type II diabetes.

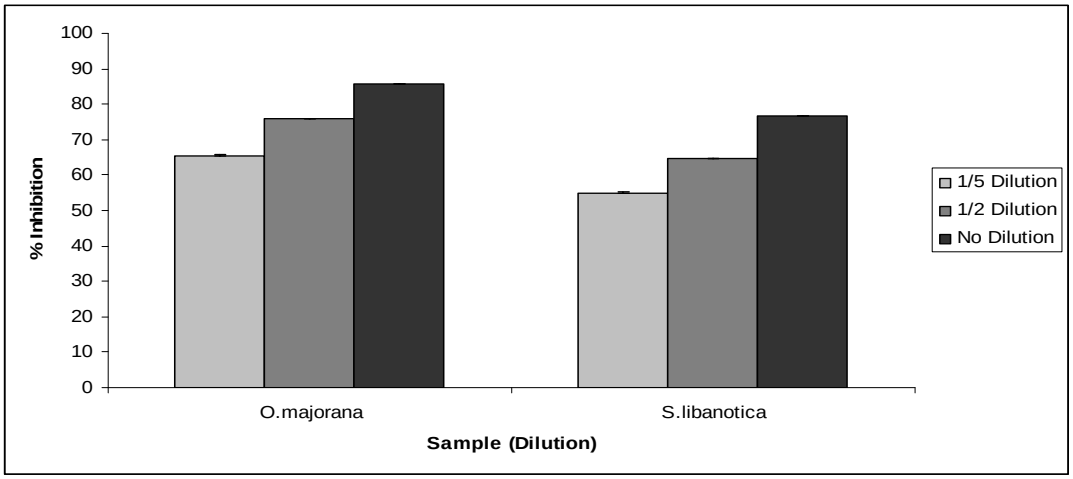

Figure 18 Changes observed in dose dependent (10 µL, 25 µL, 50 µL) % α-glucosidase inhibitory activities for aqueous extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage).

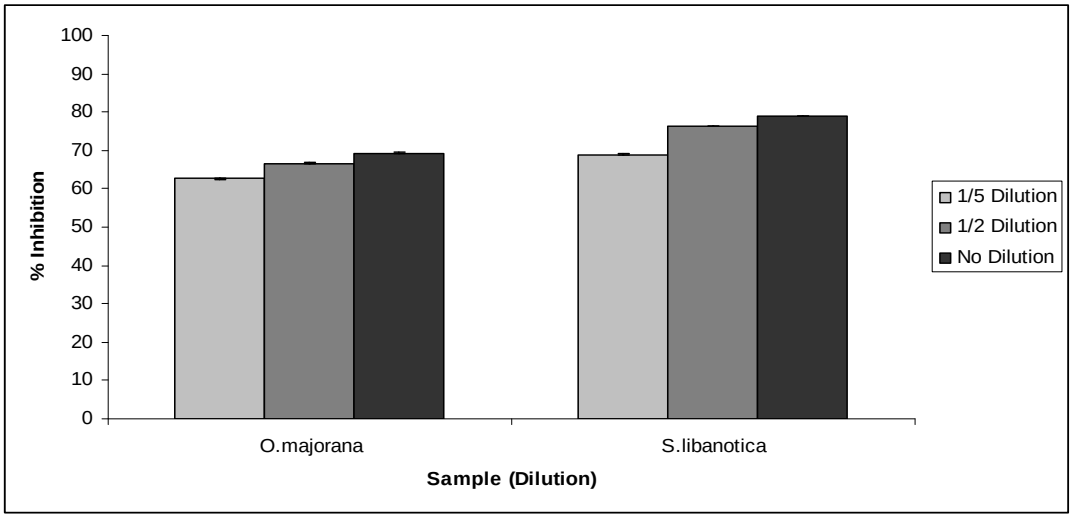

Figure 19 Changes observed in dose dependent (10 µL, 25 µL, 50 µL) % α-glucosidase inhibitory activities for ethanolic extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage).

Table 6 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Lamiaceae Family for Aqueous and Ethanolic Extracts. Analysis of Phenolic Compounds: Rosmarinic Acid, Caffeic Acid and Rutin.

Sample	Rosmarinic Acid	Caffeic Acid	Rutin
	mg/g DW	mg/g DW	mg/g DW
H2O			
Origanum majorana	27.4 ± 0.72	———	———
Salvia libanotica	39.7 ± 0.53	2.73 ± 0.14	———
ETHANOL			
Origanum majorana	5.13 ± 3.32	8.00 ± 1.15	———
Salvia libanotica	2.60 ± 0.12	———	15.0 ± 0.40

4.3.4.3 α-Amylase Inhibition

α-Amylase serves a role similar to α-glucosidase, by managing hyperglycemia linked to type II diabetes (Pinto *et al*., 2009). *In vitro* α-amylase inhibition was evaluated for ethanolic and aqueous extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage). No α-amylase inhibition was observed for either extracts of both samples. Sage and marjoram may not have some specific compounds present, which could cause it modulate α-amylase inhibition (Kwon *et al*., 2006). Though the samples did not indicate any α-amylase inhibition, but high α-glucosidase inhibition

suggests that it could still serve as a food ingredient and condiment that can be used to help manage hyperglycemia linked to type II diabetes.

4.3.4.4 ACE Inhibition

Hypertension is a risk factor for the advancement of cardiovascular diseases, which is a long term macro vascular complication of type II diabetes (Kwon *et al*., 2006). Development of natural food sources to help manage hypertension is of interest and a lower cost option. There was no ACE inhibition observed for the select species of family Lamiaceae in this study. We suspect that the reason behind not observing ACE inhibition for Lamiaceae samples could be due to the amount of sample (2.5g), which was used to prepare sample extracts. We can assume that, total phenolic content and total antioxidant activity did not influence the results obtained for ACE inhibition. This suggests that there may be other phenolic compounds or peptides not enriched in these extracts linked to ACE inhibition of sage and marjoram. (Kwon *et al*., 2006)

4.3.4.5 HPLC Analysis of Phenolic Phytochemicals

Three different major phenolic compounds were observed in ethanolic and aqueous extracts of *Origanum majorana* (Marjoram) and *Salvia libanotica* (Sage). These compounds include rosmarinic acid, caffeic acid and rutin, and amounts (mg/g DW) and their standard errors are indicated in Table 7, for both aqueous and ethanolic extracts. Rosmarinic acid observed for aqueous extracts of sage and marjoram showed a higher

content than ethanolic extracts. Rosmarinic acid content for aqueous extracts ranged from 27.4 mg/g (Marjoram) to 39.7 mg/g (Sage) of sample DW, where it ranged from 2.6 mg/g (Sage) to 5.1 mg/g (Marjoram) for ethanolic extracts. Rosmarinic acid is abundantly found in plants belonging to Lamiaceae family (Shetty, 1997; Peterson and Simmonds, 2003). Rosmarinic acid is known to provide protection against cancer and contains high antioxidant activity (Peteron and Simmonds, 2003), which may be the reason a high total antioxidant activity was observed for sage and marjoram. Presence of this particular phenolic compound in our diet as a natural food source would have the potential to manage hyperglycemia and associated obesity, preventing the incidence of type II diabetes. Additional *in vivo* studies are needed to understand the antioxidant, anti-inflammatory and anti-diabetic chemopreventive affects of rosmarinic acid from Lamiaceae plants on human health using optimized whole food extracts.

The content of phenolic compound, caffeic acid was observed in both aqueous and ethanolic extracts of the samples. The content in sage was 2.7 mg/g DW in the aqueous extracts, and marjoram 8 mg/g DW in ethanolic extracts. Caffeic acid is known to play an important part in fruits and vegetables as an antioxidant, due to which we relate its actions for potential prevention of chronic illnesses such as cardiovascular diseases and cancer. Another phenolic compound that we observed in ethanolic extracts of marjoram was rutin. Rutin content was 15 mg/g of sample DW. Phenolic flavonoids such as rutin, are commonly found in many fruits and vegetables, and are used to provide protection against development of vascular diseases (Schramm and German, 1998). This gives us some evidence on how the presence of rutin in ethanolic extract of sage could

prove to be potentially beneficial towards managing hypertension linked to type II diabetes though *in vitro* studies did not indicate such activity in this investigation.

Table 7 Phenolic Profile (mg/g of sample DW ± Standard Error) Analysis of Lamiaceae Family for Aqueous and Ethanolic Extracts. Analysis of Phenolic Compounds: Rosmarinic Acid, Caffeic Acid and Rutin.

Sample	Rosmarinic Acid mg/g DW	Caffeic Acid mg/g DW	Rutin mg/g DW
H2O			
Origanum majorana	27.4 ± 0.72	———	———
Salvia libanotica	39.7 ± 0.53	2.73 ± 0.14	———
ETHANOL			
Origanum majorana	5.13 ± 3.32	8.00 ± 1.15	———
Salvia libanotica	2.60 ± 0.12	———	15.0 ± 0.40

4.3.5 Conclusions

Main focus of this research study was to evaluate anti-hyperglycemia and antihypertensive potential of 2 select species (*Origanum majorana* and *Salvia libanotica*), of Lamiaceae family common in Near East of Asia. Samples were prepared through hot water and 12% ethanol extractions. *In vitro* functionality assays such as total soluble phenolics and inhibition assays like DPPH, α-glucosidase, α-amylase and ACE were evaluated for these select herbs to explore potential for hyperglycemic and hypertensive management. No ACE and α-amylase inhibition was observed for sage and marjoram, which could be due to absence of certain phenolic compounds in evaluated samples. α-Glucosidase inhibition for ethanolic and aqueous extracts of select samples ranged from 55% (Sage, 1/5 dilution sample) to 85.6% (Marjoram, no dilution sample). High α-glucosidase inhibition activity indicates that inclusion of herbs from Lamiaceae family could potentially help manage hyperglycemia linked to type II diabetes. Total phenolic content ranged upto 41.1 mg/g of sample DW, along with high DPPH inhibition which ranged upto almost 80%. High phenolic antioxidant activity suggests that certain phenolic compounds are present in select species, which could prove to be beneficial towards human health if included as part of food designs for a healthy diet. This research provides biochemical rationale for clinical studies on functional benefits of sage and marjoram from Lamiaceae family, which could further be applied in *in vivo* studies for development and innovation of therapeutic strategies, to prevent and manage type II diabetes.

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